VOL. XI, No. 4 NOVEMBER 1959

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

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PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

Revue internationale de nutrition des plantes, de chimie végétale, de microbiologie du sol et des maladies des plantes en rapport avec le sol

Internationale Zeitschrift für Pflanzenernährung, Pflanzenchemie, Bodenmikrobiologie und bodenbedingte Pflanzenkrankheiten

Issued under the auspices of the "Koninklijk Genootschap voor Landbouwwetenschap" (Royal Netherlands Society of Agricultural Science)

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CONTENTS: TABLE DES MATIÈRES: INHALT:

J. W. Millbank, The physiology of nitrification in Kenya highland soil . . 293

B. P. Ghildyal and *U. C. Gupta*, A study of the biochemical and microbiological changes during the decomposition of *Crotolaria juncea* (sann hemp) at different stages of growth in soil 312

J. E. Eastoe and *A. G. Pollard*, Some effects of the flowerpot on plant growth 331

P. Arambarri and *O. Talibudeen*, Factors influencing the isotopically exchangeable phosphate in soils. I. The effect of low concentrations of organic anions 343

P. Arambarri and *O. Talibudeen*, Factors influencing the isotopically exchangeable phosphate in soils. II. The effect of base saturation with sodium and calcium in non-calcareous soils 355

P. Arambarri and *O. Talibudeen*, Factors influencing the isotopically exchangeable phosphate in soils. III. The effect of temperature in some calcareous soils 364

F. Jacquin et *F. Mangenot*, Populations microbiennes des bois. III. Humification *in vitro* d'une sciure de hêtre 377

Letter to the editors, *Adam Szember*, Providing aseptically cultivated plants with water through bacteria-tight glass-filters 392

Printed in the Netherlands

Plant and Soil XI. 293-394 The Hague Nov. 1959

Plant and Soil XI, no. 4 November 1959

THE PHYSIOLOGY OF NITRIFICATION IN KENYA HIGHLAND SOIL

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INTRODUCTION

Much has been written on the nitrification process in tropical soils (Calder [1]; Caster, Martin and Buehrer [2]; Griffith [6]; Hall [7 8]; Hardy [9]; Martin and Massey [19]; and Mills [24] *inter alia*) and studies on the mineralisation of soil organic nitrogen, which almost inevitably involves the nitrifying organisms, are routine practice in most agricultural laboratories throughout the world. However, the extensive work of Lees, Quastel and their collaborators (Lees and Quastel [14 15 16 17]; Lees [12]; Hofman and Lees [11]; Lees and Simpson [18]; Quastel and Scholefield [25] *etc.*) and the pioneer investigations of Meyerhof [21 22 23], with organisms from temperate soils provides virtually the only data on the physiology and biochemistry of these important organisms. The work to be presented here represents an investigation of the physiology of the organisms responsible for nitrification in a tropical soil, with a view to establishing their similarity or otherwise to those commonly encountered in temperate latitudes.

MATERIALS AND METHODS

Materials

Soil used. The soil used in all the experiments to be described is a humic red latosol, derived from quartz trachite, which is of widespread occurrence in the high-altitude districts of Kenya. The samples, known locally as Kikuyu Red Loam, were all taken on the E.A.A.F.R.O. estate, Muguga, situated at an altitude of 6,800 ft above sea level and at latitude 1°S. approx.

After collection, the 0–6″ samples were air dried for 24 hours at room temperature (22° C) and sieved .The 4.75–1.68 mm crumb fraction (3/16″–10 mesh Greenings sieves, to B.S. 410/1943) was used.

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Methods

Soil perfusion apparatus. The soil-perfusion technique has been fully described by Lees and Quastel [15]. The perfusion apparatus used was similar to that described and illustrated by Chase [3], with the further simplification of substituting a glass cylinder and bungs for the separating funnel used as the liquid reservoir. The vacuum was produced by a rotary pump and control switch working into a reservoir of 15 l capacity. Sampling procedure of the perfusate was as described by Chase [3].

Determination of nitrate. The method adopted was essentially that described by Chase [3], with slight modifications. The procedure is as follows: An aliquot of the perfusate, diluted if necessary to contain 10 to 100 μg NO_3–N is placed in a stoppered tube, with a graduation at 25 ml. One-tenth ml hydrogen peroxide (100 vol.), and 0.1 ml calcium hydroxide solution (saturated) are added. The mixture is then evaporated to dryness at 110° C in an oven. After cooling, 0.5 ml phenol disulphonic acid reagent is added, and allowed to stand 30 min; 10 ml distilled water are then added, mixed, and the solution made up to 25 ml with sodium hydroxide solution (3*N*). The light absorption was determined in a 'Unicam' SP300 colourimeter with the standard blue filter, using a distilled-water blank carried through the analytical procedure as reference. Nitrate nitrogen was determined by reference to a calibration curve previously prepared from standard solutions.

The colour developed is a measure of the combined nitrite nitrogen and nitrate nitrogen present in the solution. When the presence of nitrite was known or suspected a separate determination was carried out and the amount found subtracted from the composite determination to derive the true nitrate concentration.

Occasionally the perfusate was not colourless; in such cases the phenol-disulphonic acid method is unsuitable. Instead, the microdiffusion technique of Conway [4] was used, allowance being made for free ammonia present.

Determination of nitrite. A suitably diluted sample of perfusate, containing 1 to 6 μg nitrite nitrogen, was made up to 6 ml with distilled water in a 25-ml calibrated stoppered tube. Six ml of 20% (v/v) acetic acid followed by 0.2 ml 50% (v/v) hydrochloric acid were added. To the mixture 1.0 ml sulphanilic acid reagent (0.75% w/v in 30% v/v acetic acid) was added, mixed thoroughly and allowed to diazotise for 5 minutes, at room temperature (approx. 22° C). 0.2 ml of 0.25% (w/v) *N*-(1 : naphthyl) ethylene diamine dihydrochloride solution, in water, was added, the mixture made up to 25 ml with distilled water and allowed to couple for 15 min at room temperature. The light absorption was determined with the Unicam colourimeter using the standard green filter, against a distilled-water blank carried through the same procedure, and nitrite-nitrogen concentration obtained by reference to a standard curve.

Estimation of methionine and histidine. These amino acids were qualitatively estimated in the perfusing solution by descending filter-paper chromatography of an appropriate volume of perfusate. The chromatograms were run on Whatman No. 1 paper, using butanol–acetic acid–water solvent (40 : 10 : 50 v/v) and developed with ninhydrin in the usual way.

ISOLATION OF ORGANISMS

The organisms responsible for nitrification were isolated by the enrichment-culture technique as modified by Meiklejohn [20] for *Nitrosomonas* sp.; and by a very similar method for *Nitrobacter* sp.

Five additions of ammonium sulphate or sodium nitrite were made to the original medium, (Medium "B" of Meiklejohn, with the relevant nitrogen source) in order to build up a population of predominantly nitrifying organisms. The precipitate in the medium was observed to agglutinate after some weeks of incubation. From this starting point, a number of subcultures were made into mineral medium "B" with the appropriate nitrogen source. At the tenth subculture, the organisms were "blown off" the particles of calcium carbonate in the medium by means of a stream of filtered and sterile CO_2 and the suspension, diluted tenfold, used to inoculate silicagel plates, the silica sol being prepared according to Smith [26] and gelled by means of the nutrient salt solution described by Meiklejohn [20].

The plates were incubated at 25° C for about 14 days, and examined with a low-power microscope. Tiny, glassy colonies were observed, about 100 μ diameter. Representative specimens were picked off by hand with a Pasteur pipette and transferred to 5 ml medium "B" in a 50-ml conical flask. The silica-gel plate was then tested for nitrite and nitrate. Only those flasks containing subcultures from plates with positive reactions were incubated. After 10 days at 25° C the flasks contained nitrite or nitrate in quantity. Part of the contents were diluted and plated on silica gel. The remainder was kept in the incubator. After 21 days the nitrite concentrations in three of the four "Nitrosomonas" medium flasks were unchanged and so it was assumed that these were free from *Nitrobacter* sp. No growth on nutrient agar was obtained either from these flasks or the nitrite medium ("Nitrobacter") flasks.

The colonies obtained from these last inoculations on silica gel were round, compact, and glassy. They did not, however, become brown on ageing. The ammonia oxidisers were non-motile, short rods or oval cells, 1.0 — 1.3 μ × × 1.8 — 2.0 μ, gram negative). The colonies were always round or oval; none of a "starfish" shape were ever observed.

The nitrite oxidisers were apparently rather smaller cells than the ammonia oxidisers. It was not possible to make exact measurements of cell size.

From these observations it is concluded that the organisms responsible for nitrification are morphologically the classical autotrophic genera, *Nitrosomonas* and *Nitrobacter*. The study of their physiological characteristics follows.

RESULTS

Shape of the build-up curve of nitrate

A series of nitrification curves in which 40 g airdried soil taken from various sites on the Muguga estate were perfused at approx.

22° C with 100 ml 0.01 *M* ammonium sulphate solution (contains 280 μg NH_4-N/ml) is shown in Figure 1. Noticeable features are the very slow rate of nitrification except with the "potting" soil from the plant nursery, and the inability of the soil from an arable, fallow field to nitrify more rapidly than that from beneath a dense cover of Kikuyu grass (*Pennisetum clandestinum*). No reaction occurred at all with sterilised soil.

Figure 2 shows the effect of addition of a number of mineral nutrients to soil from three different locations. After the characteristic lag phase, the rate of nitrification increases rapidly, and then tails off when conversion of the added ammonia is virtually

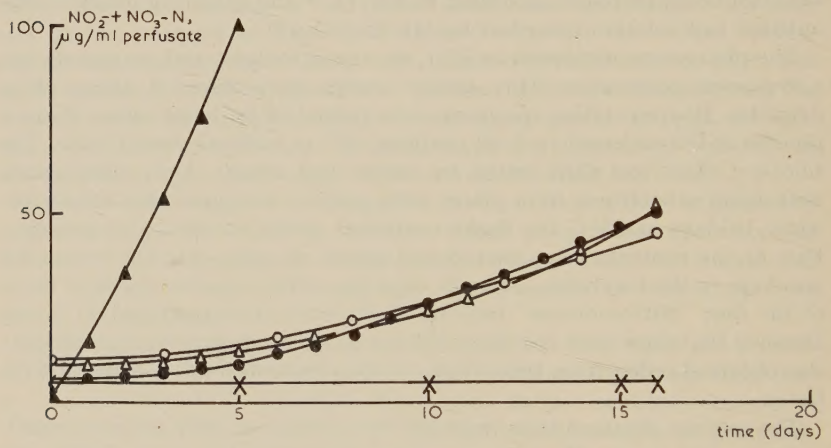

Fig. 1. Variation in nitrite + nitrate-N concentration with time of perfusion of various soils from E.A.A.F.R.O. estate, with no additions. Perfusion units contained 40 g air-dried soil, perfused with 0.01 *M* ammonium sulphate.
Symbols: × Sterilised control soil.
● Kikuyu grass soil.
○ Wattle plantation soil.
△ Arable field soil.
▲ Nursery potting soil.

complete. The curve is similar to those found in studies of proliferating cells, and it would appear that the process of nitrification under these conditions is being brought about by rapidly multiplying cells. The constituent substance of the added mixture primarily responsible for the very marked improvement in nitrification was found to be calcium carbonate. Other ions, including

those of trace elements, had no apparent beneficial effect. Measurements of the reaction of the perfusing solution showed that all the samples of soil, unlike those used by Lees and Quastel [15 16] had only a very limited capacity to buffer the increase in acidity that accompanies the oxidation of ammonia to nitrate. In the absence of any added neutralising material, the pH of the perfusing solution rapidly falls to 5 or below, and henceforward nitrification becomes very slow or ceases altogether. Calcium carbonate buffers the pH to approx. 7.4 and nitrification proceeds rapidly.

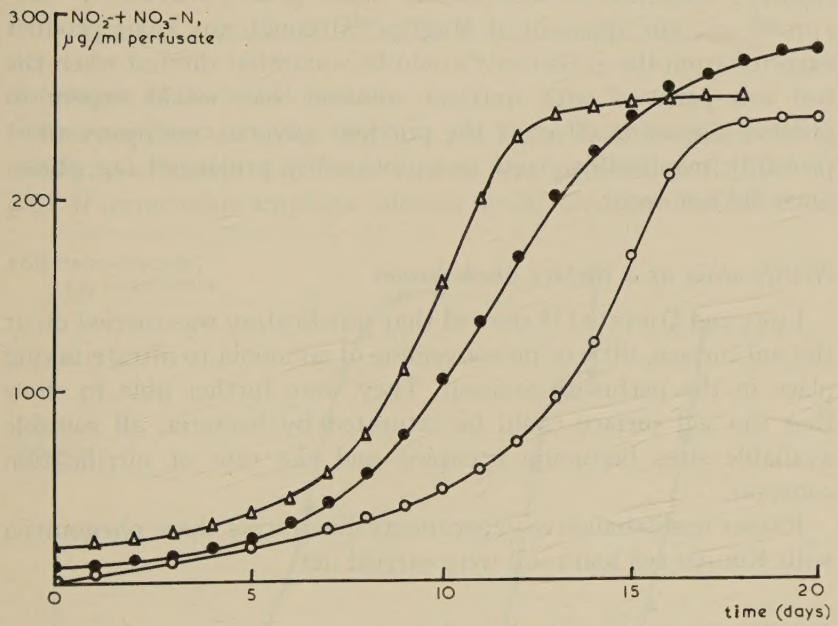

Fig. 2. Variation of nitrite + nitrate-N with time of perfusion in three soils from E.A.A.F.R.O. estate, with additions. Perfusion units contained 40 g air-dried soil, 0.5 g calcium carbonate, perfused with 100 ml 0.01 *M* ammonium sulphate solution, containing K_2HPO_4 (0.175 g) $MgSO_4$ (0.250 g) and trace elements.

Symbols: △ Kikuyu grass soil.
● Wattle plantation soil.
○ Arable field soil.

Extensive further experiments which are not reported here in detail were undertaken to determine whether the pattern of nitrification in Kikuyu red loam soil depended on the nature of the plant

cover or land use. It was particularly noticeable that with the sole exception of the nursery potting soil mentioned above no variation occurred in the normal pattern of 8 to 10 days lag phase (depending on the initial soil-moisture content) prior to the commencement of cell proliferation. The maximum rate of nitrification was also consistent throughout. Soils examined were 0–6″ samples taken from below Kikuyu grass sward, a lucerne variety trial, a wattle plantation, (*Acacia* hybrid sp.), a maize plantation, *Cynodon* (Stargrass) sward and arable fallow fields in various locations. The reported inhibition of nitrification under grass (Stiven [28], Theron [29]) was not apparent at Muguga. Although any toxic product excreted from the grass roots would be somewhat diluted when the soil was perfused with nutrient solution, one would expect to observe a residual effect of the previous adverse conditions, most probably manifesting itself in a noticeably prolonged lag phase. Such did not occur.

Nitrification as a surface phenomenon

Lees and Quastel [16] showed that nitrification was carried on at the soil surface, little or no conversion of ammonia to nitrate taking place in the perfusing solution. They were further able to show that the soil surface could be saturated by bacteria, all suitable available sites becoming occupied and the rate of nitrification constant.

Rather less exhaustive experiments illustrating these phenomena with Kikuyu red loam soil were carried out.

Nitrification in the perfusing solution

The rate of nitrification in the perfusing solution was studied by removing the soil from a rapidly nitrifying system, and continuing the perfusion over sterile sand. The nitrate and ammonia concentrations were determined initially and one week after replacement of the soil by sand. The results are shown in Table 1. It is evident that virtually no nitrification is carried on in the perfusing solution, the small change in nitrate concentration being accountable by the particles of soil in the lower reservoir of the perfusion unit, that had penetrated the filter pad at the beginning of the experiment.

TABLE 1

Nitrification in the perfusing solution.
40 g soil were placed in each of four perfusion units, 0.5 g $CaCO_3$ added, and perfused with 100 ml 0.01 *M* $(NH_4)_2SO_4$ for 9 days at 22° C. After this period the soil was removed from two of the perfusion units and replaced with sterile sand, the others being left intact. Perfusion was continued in all four units.

Days after removal of soil	Nitrite- + Nitrate-N produced μg/ml perfusate	
	In perfusers where soil was untouched	In perfusers where soil was replaced with sterile sand
0	82 88	76 71
7	230 230	81 78

Saturation of the soil by bacteria

Forty grams air-dried Kikuyu red loam soil, to which 0.5 g calcium carbonate had been added, was perfused with 100 ml 0.01 *M* ammonium sulphate solution for 20 days, until nitrification

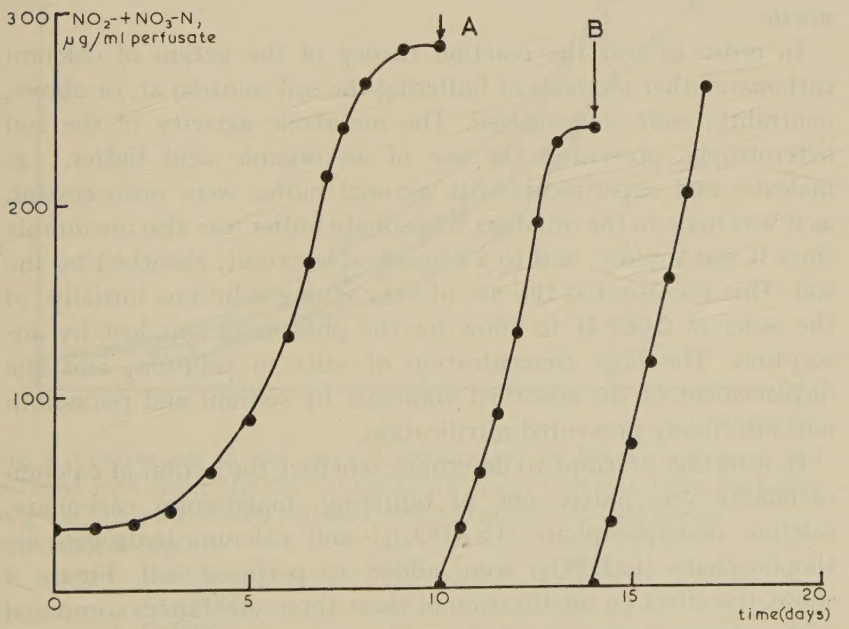

Fig. 3. Effect of washing and re-perfusing Kikuyu red loam soil. Soil (40 g) was perfused with 0.01 *M* $(NH_4)_2SO_4$; at points A, B, column was washed with one litre tap water, $CaCO_3$ replenished, and fresh $(NH_4)_2SO_4$ solution re-perfused. For strengths of solutions, see text.

was complete (Point "A"). The soil column was then washed with one litre tap water to remove the nitrate formed, the calcium carbonate replenished with 0.25 g, 100 ml fresh ammonium sulphate solution added, and perfusion re-commenced. Reference to Figure 3 will show that the characteristic lag phase had been eliminated and nitrification was proceeding at a constant rate. When all the added ammonia had been converted (Point "B") the column was again washed and 100 ml 0.02 *M* ammonium sulphate added, with further calcium carbonate. This second quantity was again nitrified immediately and rapidly, at the same rate.

These experiments show that proliferation has not occurred beyond a maximum evidently permitted by the soil surface and that the organisms are themselves attached quite strongly to the soil particles, and are not free living in the soil solution to any appreciable extent.

The use of alternative buffers, and the transitory accumulation of nitrite

In order to test the reaction theory of the action of calcium carbonate other methods of buffering the soil solution at, or above, neutrality, were investigated. The metabolic activity of the soil heterotrophs prevented the use of an organic acid buffer, *e.g.* maleate, and experiments with veronal buffer were unsuccessful, as it was toxic to the nitrifiers. Phosphate buffer was also unsuitable since it was rapidly, and to a considerable extent, absorbed by the soil. This necessitated the use of very strong solutions initially, of the order of 0.067 *M* to allow for the phosphate ions lost by adsorption. The high concentration of salts in solution, and the displacement of the adsorbed ammonia by sodium and potassium ions effectively prevented nitrification.

In a further attempt to determine whether the action of calcium carbonate was purely one of buffering, magnesium carbonate, calcium orthophosphate [$Ca_3(PO_4)_2$] and calcium hydrogen orthophosphate ($CaHPO_4$) were added to perfused soil. Figure 4 shows the effect on nitrification of these three substances compared with calcium carbonate. It is evident that $CaHPO_4$ is least effective, and reference to Table 2 will show that the pH of the perfusing solution was not maintained. As the pH fell, noticeably after day 13, the rate of nitrification diminished considerably. It is

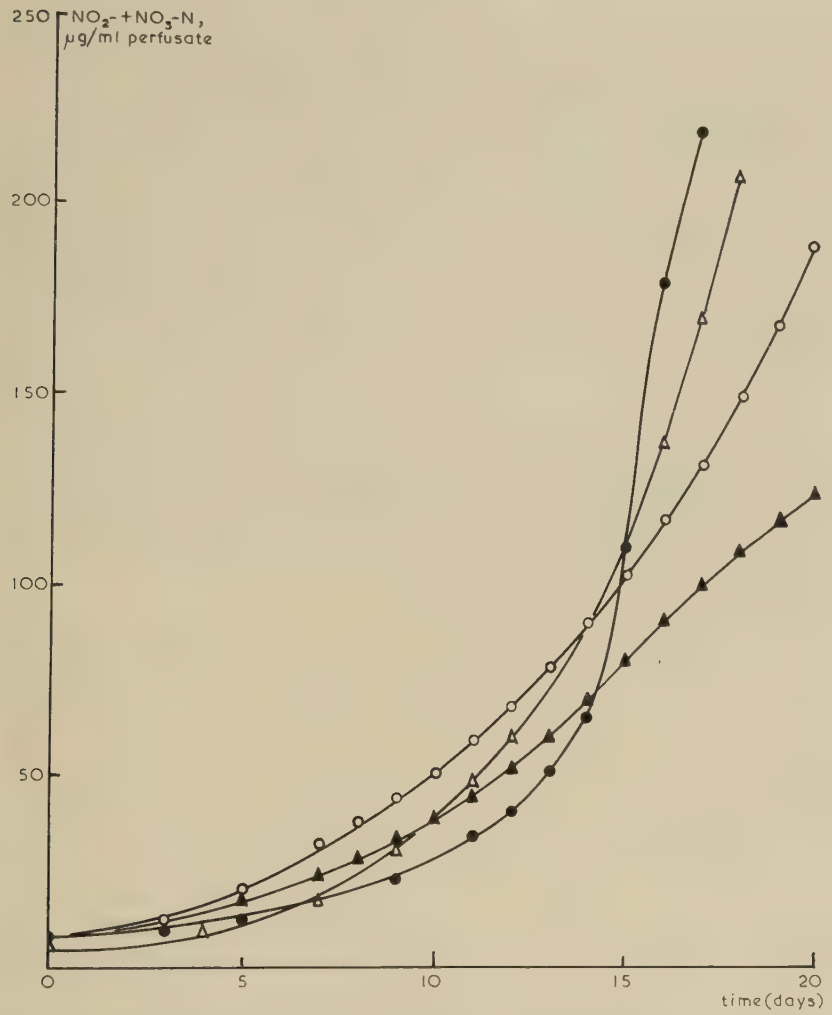

Fig. 4. Comparison of the rate of nitrification in Kikuyu red loam soil buffered with either $MgCO_3$, $CaCO_3$, $Ca_3(PO_4)_2$ or $CaHPO_4$. Units contained 40 g soil, 0.5 g alkali, and perfused with 100 ml 0.01 *M* $(NH_4)_2SO_4$.
Symbols: ● $MgCO_3$.
△ $CaCO_3$.
○ $Ca_3(PO_4)_2$.
▲ $CaHPO_4$.

considered that the effect on nitrification of these basic substances is entirely due to maintenance of neutral to alkaline conditions, and not the supply of an ion lacking from the soil.

TABLE 2

Buffering effect of various solid alkalies on pH of perfusate
Perfusion units containing 40 g soil to which 1.0 g alkali had been mixed, were perfused with 100 ml 0.01 *M* $(NH_4)_2SO_4$. The pH of the perfusate was determined electrometrically from a 10-ml sample of the perfusate which was subsequently returned to the perfusing system

Alkali added	Days									
	1	2	4	6	8	10	11	13	14	17
$CaCO_3$	7.6	7.6	7.7	7.5	7.7	7.7	7.4	7.3	7.2	7.2
$MgCO_3$	8.6	—	8.5	—	8.7	—	8.6	—	8.5	8.6
$CaHPO_4$	6.8	6.3	6.2	6.3	6.2	6.0	5.8	5.2	5.5	5.2
$Ca_3(PO_4)_2$	6.2	—	—	—	6.0	—	6.0	—	5.8	5.6

Lees and Quastel [15], Chase [3] and Stevenson and Chase [27], for example, found the accumulation of nitrite under the normal aerated conditions of perfusion with ammonium sulphate solution, to be negligible. Experiments with Muguga soil have shown that a

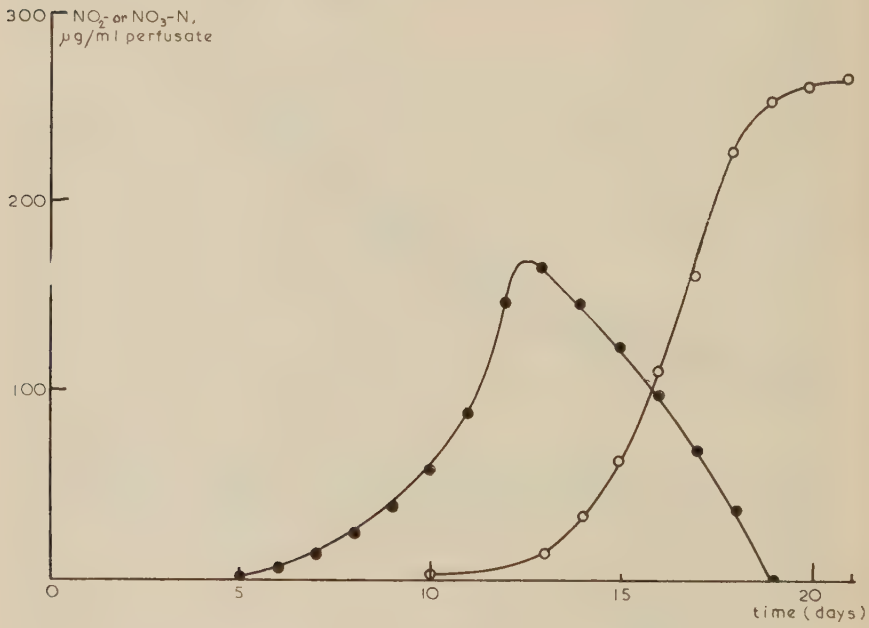

Fig. 5. Individual variation in nitrite-N and nitrate-N with time when 40 g Kikuyu red loam soil is perfused with 100 ml 0.01 *M* $(NH_4)_2SO_4$ in the presence of 1.0 g $CaCO_3$.
Symbols: ● Nitrite nitrogen.
○ Nitrate nitrogen.

marked accumulation can build up under certain conditions. Such conditions can well occur in agricultural practice.

Figure 5 shows a typical result when 1.0 g calcium carbonate is added to 40 g soil and perfused with 100 ml 0.01 *M* ammonium sulphate. No nitrite appears at all in the control, to which no calcium carbonate has been added. The pH of the experimental unit remains sensibly constant at 7.4; that of the control rapidly falls to approx. 5, whereupon nitrification ceases.

The effect of diminishing additions of calcium and magnesium carbonates on nitrite accumulation was investigated, and the results shown in Table 3.

TABLE 3

The effect of addition of differing quantities of alkaline buffers on the pH of the perfusate and the accumulation of nitrite

Units containing 40 g soil mixed with various quantities of $MgCO_3$, $CaCO_3$, and $Ca_3(PO_4)_2$ were perfused with 100 ml 0.01 *M* $(NH_4)_2SO_4$. pH and nitrate-N were measured at intervals on samples of the perfusate

Substance added	Amount	pH		Max. nitrite concn. in perfusate (μg/ml)
		Day 0	Day 12	
$CaCO_3$	0.2 g	7.5	5.7	52
	0.5 g	7.5	6.8	145
	1.0 g	7.5	7.4	170
$MgCO_3$	0.16 g	7.5	6.2	44
	0.5 g	8.5	8.5	240
	1.0 g	8.6	8.5	240
$Ca_3(PO_4)_2$	0.5 g	6.9	5.5	0
None (control)	—	5.8	4.9	0

The minimum amounts of alkali used were the quantities calculated to neutralise the acid formed when all the ammonia was nitrified. Measurements of pH suggested that nitrite accumulation was closely related to the pH of the perfusing solution. This was particularly noticeable with magnesium carbonate, the nitrite accumulation being very pronounced and the pH of 8.6 over a unit higher than when calcium carbonate was used. Calcium phosphate ($Ca_3(PO_4)_2$) on the other hand, gave no nitrite accumulation, the initial pH being appreciably lower than with calcium carbonate.

When a bacterially saturated soil is washed, calcium carbonate

added, and re-perfused with 0.01 *M* ammonium sulphate (*e.g.* Figure 3) no nitrite is found to accumulate. The effect of perfusing a magnesium carbonate-fortified, bacterially saturated soil with sodium nitrite solution, is shown in Figure 6. The conversion to

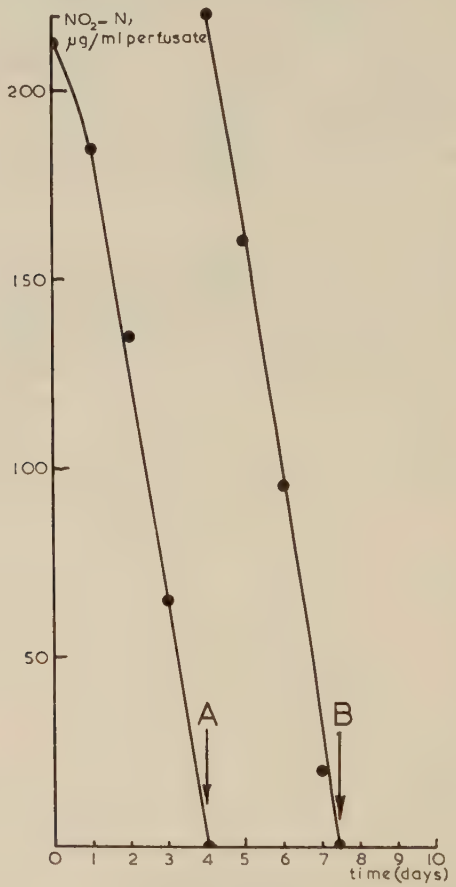

Fig. 6. Change in nitrite-N with time when a bacterially saturated soil is perfused with two successive batches of sodium nitrite solution, at pH 8.7. Nitrate-N concn. at time A: 210 µg/ml, at time B: 215 µg/ml.

nitrate, at pH 8.7, is immediate, quantitative, and rapid. It would seem that the alkaline conditions are not inhibiting the action of *Nitrobacter* in converting nitrite to nitrate, but retarding its rate of proliferation relative to *Nitrosomonas*, and thus giving rise to a

transitory accumulation of nitrite. The use of calcium carbonate gives an exactly similar result.

To demonstrate this reduction in rate of proliferation, the experiment illustrated by Figure 7 was carried out. A series of perfusion units were set up each containing 0.02 *M* sodium nitrite solution, (contains 280 μg NO_2-N/ml) and 40 g Kikuyu red loam soil, which had not been enriched with organisms. To the soil in the

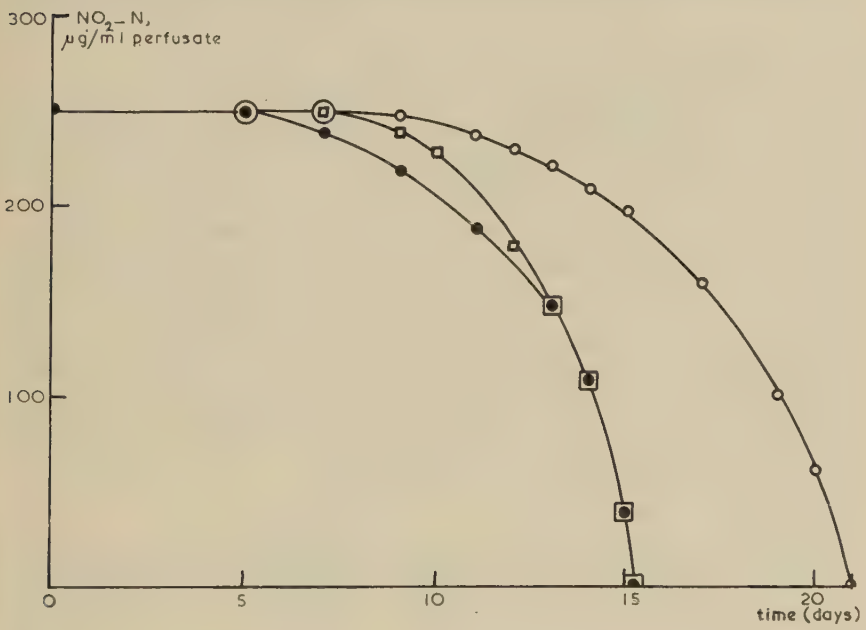

Fig. 7. Change of nitrite-N concentration in soils perfused with sodium nitrite solution (0.02 *M*) and held at various pH ranges.
Symbols: ○ pH 8.8.
□ pH 7.4—7.7.
● pH 6.8—7.0.

units was added a range of quantities of calcium or magnesium carbonates, thus giving rise to perfusing solutions of various pH's. A control, to which magnesium sulphate instead of magnesium carbonate was added, was also prepared. It was expected that with increasing alkalinity the lag phase of growth, manifested by little or no change in nitrite concentration of the perfusate, would be of increased duration. Figure 7 shows that this expectation was

borne out in practice. Following from this, an examination of the rate of oxidation of ammonia to nitrite by fresh soil in perfusion units showed it to be unaffected by pH within the range 6 to 8.8. Hence, when ammonium sulphate solution is added to a fresh soil, the population of ammonia oxidisers builds up in the normal way irrespective of pH within a wide range, and is therefore unaffected by the presence of calcium or magnesium carbonates. The nitrite oxidisers, on the other hand, are greatly affected by pH, conditions above 7.5 to 7.7 giving rise to a very reduced rate of proliferation and a transitory accumulation of nitrite.

These results agree well with those of Caster *et al* [2] who, in an exhaustive investigation of some Arizona soils first showed conclusively that the hitherto widely held belief that nitrite does not appear when ammonia is nitrified in soil was by no means axiomatic, and quoted the 'threshold value' of pH 7.7 for nitrite oxidation, above which the *Nitrobacter* population builds up extremely slowly. Nitrite accumulation has also been found to occur in soils to which large amounts of calcium carbonate have been added. (Drouineau, Gouny and Lefèvre [5]). Lees [13] (page 93) also quotes a pH optimum of 7.7 for a stable population of *Nitrobacter*, but it is also evident that the organisms can oxidise nitrite rapidly at pH 8.5 once a population is estabilished.

The effect of inhibitors on nitrification in Muguga soil

The procedure adopted was to add the inhibitor to the perfusion liquid and compare the production of nitrate and/or nitrite with that in a control unit.

(a) Chlorate. Since nitrite normally accumulates to an appreciable extent at first when Muguga soil is perfused with ammonium sulphate, any effect of chlorate would be to a large extent masked if un-enriched soil were to be used. Soil which had been enriched with an active nitrifier population by addition of calcium carbonate and pre-perfusion with 0.01 M ammonium sulphate was therefore used.

Figure 8 shows the effect of 10^{-3} M and 5×10^{-4} M potassium chlorate. Complete inhibition of *Nitrobacter* activity was secured, which parallels the results of Quastel and Scholefield [25].

(b) Allyl thiourea. The effect of 10^{-3} and 10^{-4} M allyl thiourea on nitrification in bacterially enriched Muguga soil is

shown in Figure 9. The inhibition of nitrification was complete until the chelating agent had evidently been broken down by the soil heterotrophs, at which point nitrification commenced.

(c) Histidine and methionine.

i. Histidine. Forty grams Kikuyu red loam soil + 0.5 g calcium carbonate were perfused with 100 ml of a solution containing ammonium sulphate and histidine, both substances at a concentration of 0.01 *M*. A qualitative assay of histidine in the perfusing

Fig. 8. Effect of ClO_3^- on oxidation of ammonia in bacterially enriched Kikuyu red loam soil. Soil (40 g) was perfused with 100 ml 0.01 *M* $(NH_4)_2SO_4$ solution, containing zero, 10^{-3}, and 5×10^{-4} *M* $KClO_3$.

Symbols: △ Nitrate N in control (zero $KClO_3$).
● Nitrite N, with 10^{-3} *M* $KClO_3$.
○ Nitrite N, with 5×10^{-4} *M* $KClO_3$.
□ Nitrite N in control (zero $KClO_3$).

solution was made by removing 0.05 ml daily and chromatographing on filter paper. For comparison, a 'marker' mixture of six amino acids including histidine, was run. After perfusion for 24 hours the histidine content of the solution was almost zero, and at 48 hours none remained. Subsequently, the course of nitrification was normal.

It was quite evident that the heterotrophic population had metabolised the amino acid. The experiment was not continued.

ii. Methionine. An exactly similar experiment was carried out with methionine. Qualitative chromatographic assay showed that in five days the methionine had disappeared, and the smell of mercaptans became evident (see Lees[12]). These were clearly toxic to the nitrifiers as the normal course of nitrification did not begin until 20 days after the commencement of perfusion. By this

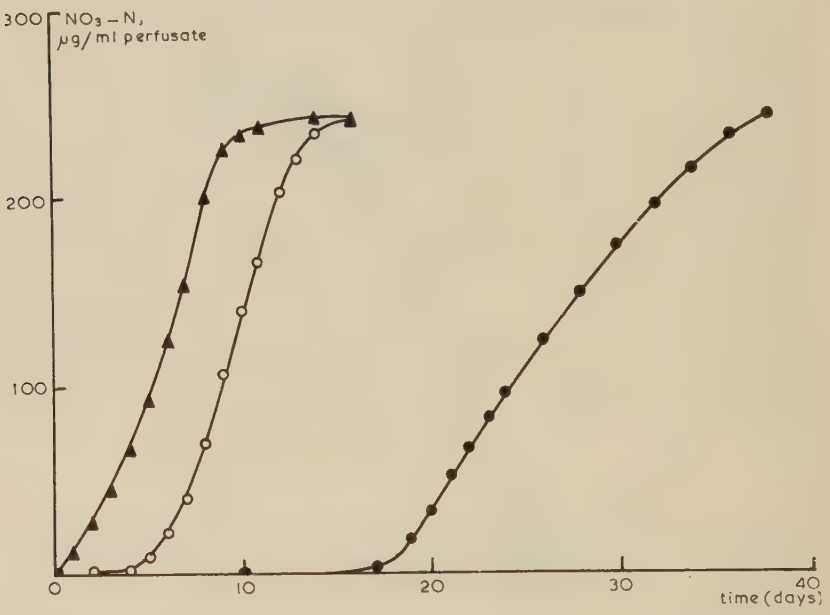

Fig. 9. Effect of allyl thiourea on oxidation of ammonia by Kikuyu red loam soil. Perfusers contained 40 g bacterially enriched soil, perfused with 100 ml 0.01 *M* $(NH_4)_2SO_4$ solution.

Symbols: ▲ Control – no allyl thiourea.
○ 10^{-4} *M* allyl thiourea.
● 10^{-3} *M* allyl thiourea.

time the odour of mercaptans had vanished, evidently indicating their breakdown. No abnormalities were observed over the subsequent course of nitrification.

(d) Extracts from Kikuyu grass (*Pennisetum clandestinum*) This grass is known to contain up to 0.3% dry weight of methionine and it has been suggested that the apparently low rate of nitrifi-

cation beneath the grass cover could be due to the toxic effects of breakdown products of the methionine after its release into the soil by plant decay, or excretion (Hesse [10]). An extract of the grass was accordingly prepared by maceration with an equal weight of water in an "Ato-Mix" homogeniser. The "brei" was strained through muslin to remove large particles and used diluted to one-half. No noticeable inhibitory effect was observed when it was perfused either through soil from a Kikuyu grass sward or from under a wattle plantation. It is considered that the low rate of nitrification observed by Hesse was due to failure to allow for the low buffering capacity of the soil. At no time in this investigation was any abnormality observed with soil from Kikuyu grass plots.

DISCUSSION

The experimental results quoted above make it evident that at least in the red humic latosols of the Kenya highlands nitrification is a biological process, carried on by autotrophic bacteria morphologically closely resembling if not identical to the classical genera *Nitrosomonas* and *Nitrobacter*. Physiologically, their pH relations strong affinity for soil particles, reaction to inhibitors, and attribute of saturating a soil with a finite population again provides strong evidence of their close similarity to those organisms studied in temperate latitudes.

The lack of any differentiation in the pattern of build up of population in the soils used is striking. The only influential factor appeared to be soil-moisture content, which reduced the lag phase by about two days, after a long period of rainy weather. Grass cover, relative to arable and fallow soil had no observable effect on the initial lag phase. It is therefore considered unlikely that any toxic substances were being released from the grass roots; if such a substance was being released it was evidently being rapidly destroyed by the soil heterotrophs.

Nitrite accumulation when the conditions were made suitable for rapid nitrification was particularly sytriking. The inability of the soil to buffer the acid produced when ammonium sulphate is added and nitrified is a considerable disadvantage. Liming is therefore essential to enable the nitrifier population to deal with the added ammonia, and it is quite evident that a delicate balance

must be struck between the quantities of acidogenic fertiliser applied and neutralising lime added if a serious build up of nitrite in the soil is to be avoided. As a first approximation, at least sufficient calcium carbonate to neutralise the acid formed when the ammonia is oxidised should be added, but it is clearly desirable to determine previously the ability of any particular soil to resist change in pH, since greatly reduced amounts of lime may well be needed.

SUMMARY

1. The organisms responsible for the conversion of ammonia to nitrate in a soil widely distributed in the high altitude (over 6000′) areas of Kenya have been investigated, both morphologically and physiologically, using the soil-perfusion technique.

2. Morphologically, apart from motility, they closely resemble the classical genera *Nitrosomonas* and *Nitrobacter*. They are short rods, gram negative, nonmotile, with round, compact and glassy colonies.

3. Physiologically, they also show close resemblance to the temperate forms. Nitrification is carried on only at the soil surface; the surface can be saturated with bacteria, and the bacteria adhere strongly to the soil particles.

4. A soil which has been enriched and saturated with organisms oxidises nitrite and ammonia at a constant rate, with no initial lag phase. The pH is not critical over a wide range.

5. The build-up of a population on additions of ammonium sulphate to untreated soil is slow, due to the onset of acid conditions created by the oxidation of ammonia to nitrite. Addition of alkaline substances to combat this gives rise to transitory accumulations of nitrite, caused by differential pH optima for the proliferation process of the two organisms involved.

6. Chlorate and allyl thiourea are powerful inhibitors of nitrification in the soils studied. Methionine is broken down to mercaptans, which are inhibitory; Histidine is eliminated by the soil heterotrophs and so is not inhibitory.

ACKNOWLEDGEMENT

The author is indebted to Mrs. M. A. Ledger for technical assistance during part of this work.

Received March 7, 1959

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A STUDY OF THE BIOCHEMICAL AND MICROBIOLOGICAL CHANGES DURING THE DECOMPOSITION OF *CROTOLARIA JUNCEA* (SANN HEMP) AT DIFFERENT STAGES OF GROWTH IN SOIL

by B. P. GHILDYAL * and U. C. GUPTA **

The decomposition of plant materials under tropical conditions has been fairly extensively studied in the preparation of composts and artificial organic manures by a number of Indian workers [1 8 11 13]. The nature of the various biochemical and microbiological processes underlying the decomposition of green manures in the soil, however, has not been studied in detail. Bal [4] followed the decomposition of some common green-manuring plants of different stages of growth in the Black Cotton soils of Madhya Pradesh; Joshi [14] investigated the comparative manurial value of the whole plant and different parts of green manure; Daji [7] also studied the decomposition of green manures in soil and Shrikhande [20] used tea leaves as green manure in the biological decomposition studies.

In the foregoing studies, attention has been paid only to the changes undergone by the various plant constituents in the soil and not to the agencies responsible for these changes. For understanding the nature of the decomposition processes, it is essential to know the relative abundance of various groups of micro-organisms at a particular stage of decomposition.

In the present study, therefore, both the biochemical and microbiological changes, during the decomposition of sann hemp in the soil were studied. As microbiological activity is considerably influenced by environment and age of the plant, the course of

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decomposition of the plants, harvested at various stages of growth, was followed under optimum laboratory conditions and also under natural field conditions.

EXPERIMENTAL TECHNIQUE

Crotolaria juncea (sann hemp), the common green-manure crop of India, was used in the experiments. The plants were allowed to grow for 3, 4, 5, and 7 weeks, uprooted and the whole plant including roots cut to small pieces at various intervals. The chopped plant material was mixed with the soil at the rate of 5 g of air-dry material (containing about 30 to 40 per cent moisture) per 100 g of air-dry soil. The mixture was kept in the Pyrex beakers, and distilled water was added so as to increase the moisture content of the soil to 50 per cent of its water-holding capacity. The loss in moisture was frequently made up by the addition of distilled water.

Several sets of these treatments were incubated at 30° C in such a way that one set for each treatment was removed for analysis after 15, 45, 75, and 120 days of decomposition. The green-manure crop, after 7 weeks of growth, was ploughed down in the field and a representative soil sample was removed from the field at regular intervals for analysis.

Table 1 gives analysis of the soil and Table 2 composition of sann hemp plants at various stages of growth.

TABLE 1

Analysis of the soil used for the decomposition study of *Crotolaria juncea* plants	
a) HCl extract	
i) P_2O_5	0.120%
ii) K_2O	0.502%
iii) CaO	2.720%
iv) MgO	1.127%
v) R_2O_3	7.660%
b) Acid insolubles	81.530%
c) Organic carbon	0.632%
d) Total nitrogen	0.065%
e) Ammoniacal nitrogen *	14.0 ppm
f) Nitric nitrogen *	37.0 ppm
g) pH	7.8
h) Mechanical composition	
i) Coarse sand	0.92%
ii) Fine sand	56.82%
iii) Silt	25.30%
iv) Clay	15.30%

* At the time of sampling.

TABLE 2

The chemical composition of the sann hemp (*Crotolaria juncea*) plants at various stages of growth as per cent on oven-dry basis

Period of growth	3 weeks	4 weeks	5 weeks	7 weeks
Average height of plants	16″	33″.	48″	58″
Moisture in fresh green samples	88.25	83.25	80.44	76.10
Water-soluble organic matter	34.53	20.85	16.92	14.12
Organic carbon	50.90	53.26	53.39	56.76
Total nitrogen	3.49	3.10	2.71	1.92
Cellulose	25.62	33.08	37.92	40.24
Pentosans	13.32	14.96	16.73	18.92
Lignin	14.31	17.62	19.20	21.18
Ether extract	2.93	2.24	1.61	1.07
Ash	11.09	9.99	9.27	7.08

METHOD OF ANALYSIS

The following determinations were made:

Moisture was estimated by oven-drying the sample to constant weight at 105° C.

Total nitrogen was determined by the modified Kjeldahl's method [3].

Ammoniacal and *nitric nitrogen* were determined by Richardson's modification of Olsen's method (Piper [17]).

Organic matter: Organic carbon was determined by the modified Walkley and Black's method [26] and organic matter figures obtained by multiplying organic carbon by 1.724.

Water-soluble organic matter: 25 g of soil was shaken with 125 cc of water and 50 cc was evaporated to dryness and ignited, the loss in weight represented the water-soluble organic matter.

Pentosans were determined by the standard phloroglucide method [3].

Lignin was estimated by Norman's method [15].

Cellulose was determined by extracting the sample with Switzer's reagent (Daji [6]).

pH determinations were made by Kuhn's colorimetric method (Piper [17]).

Ether extract: 1 g of the material was extracted with petroleum either (B.P. 40° C to 60° C) in a Soxhlet apparatus. The extract was evaporated on the water bath and dried to constant weight.

Number of micro-organisms was determined by the plate count method using sodium albuminate agar for total bacteria, nitrate-sucrose agar for actinomycetes and ammonium nitrate-sucrose agar for fungi (Fred and Waksman [10]).

RESULTS AND DISCUSSION

Chemical composition of plants

The composition of sann hemp plants at various stages of maturity (Table 1) shows that with maturity the mineral matter (ash), fats

and waxes, total nitrogen and water-soluble organic matter decrease, while pentosans, cellulose and lignin increase.

Micro-organisms

i) Bacteria: When the green manure was incorporated in the soil a steep rise in the number of bacteria was observed. As is evident from the Fig. 1, the peak number of bacteria varying from 138 to 208 million

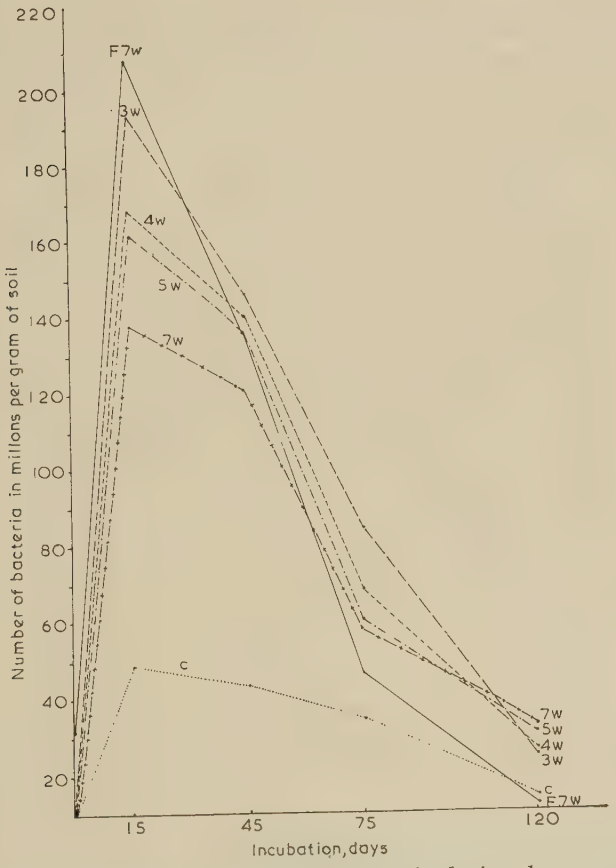

Fig. 1. Fluctuations in the number of bacteria during decomposition.

per g of soil was reached within a period of 15 days. The number of bacteria decreased gradually as the maturity of the plants increased, *i.e.* as the plants got poorer in water-soluble organic matter and nitrogen. Already after 15 days the bacterial number had decreased

rapidly. Only towards the later stages of decomposition, the number of bacteria was higher in the mature plant treatments than in the immature ones, probably due to the gradual conversion of the complex substances into simple ones through the activity of fungi and actinomycetes. Under field conditions the fall in the bacterial number was as speedy as the rise and reached a minimum of 11 millions equal to the control in 120 days, apparently due to unfavourable conditions.

ii) Actinomycetes: The number of actinomycetes in all treatments increased steadily (Fig. 2) and reached a maximum of

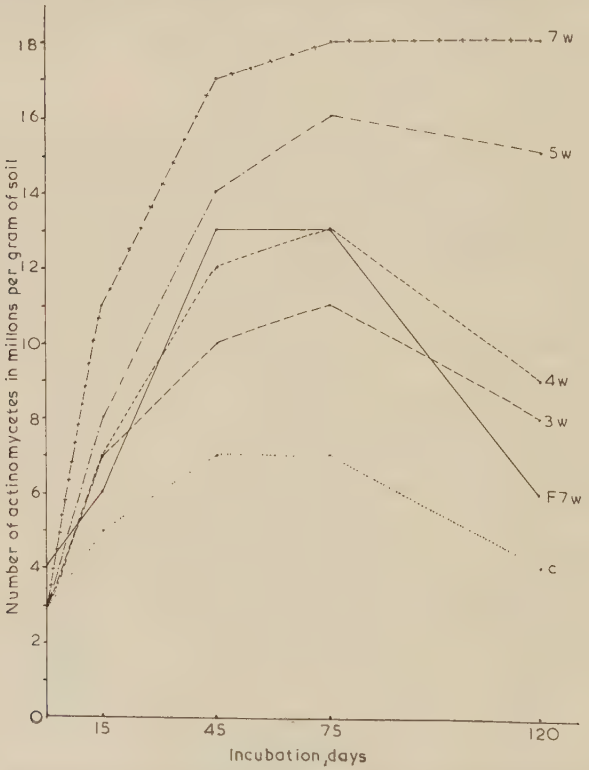

Fig. 2. Fluctuations in the number of actinomycetes during decomposition.

18 million with 7-week plants and remained later more or less constant. In the case of immature plants of 3 weeks or 4 weeks, the number decreased after a period of 75 days. The number of actinomycetes varied similarily under field conditions. The number reached was not high.

iii) Fungi: Fungi also increased rapidly (Fig. 3) under laboratory conditions and attained a peak number of 50,000 within 45 days with 7-week plants and subsequently remained nearly constant. Under field conditions, however, fungi reached a maximum number of 51,000 in 45 days and decreased subsequently rapidly.

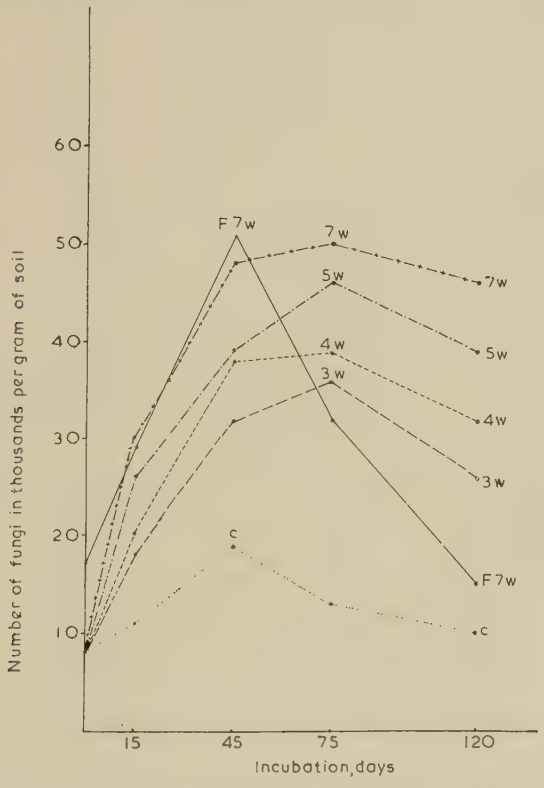

Fig. 3. Fluctuations in the number of fungi.

Nitrogen transformations

From Figure 4, it appears that the ammonification of plant material was not rapid and the maximum ammoniacal nitrogen – 34 to 36 ppm – accumulated only after a period of 75 days and that only with 4-week and 5-week plants, while ammoniacal nitrogen remained constant with 3-week plants and increased slightly in the beginning with 7-week plants. On the other hand most of nitrogen seems to have been nitrified within 45 days with 3- and 4-week

plants (Figure 5). A maximum of 365 ppm nitric nitrogen accumulated after 45 days with 3-week plants.

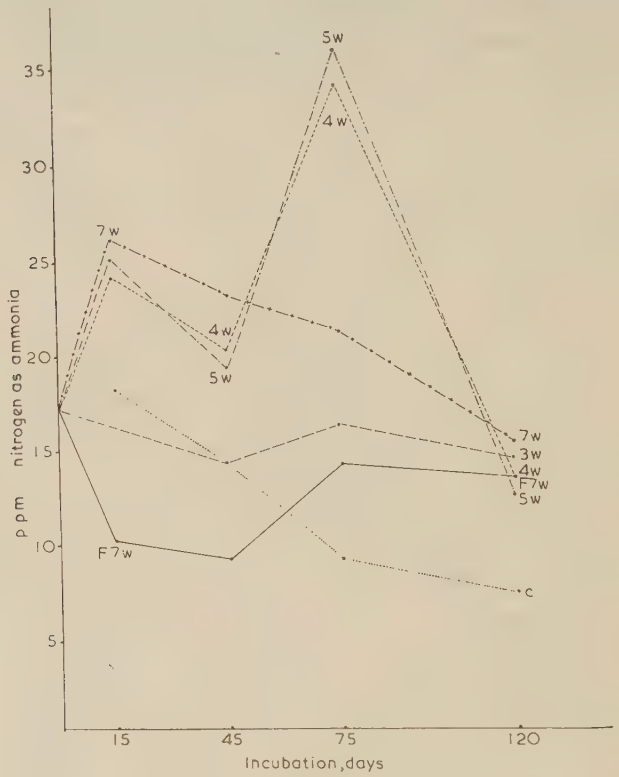

Fig. 4. Ammonia formation during decomposition.

In view of the considerable increase in nitrates with a corresponding steep rise in bacterial number and a small ammonia content and a low number of fungi, it seems that ammonification and nitrification of immature plant tissues were mostly brought about by bacteria. As 7-week plants were comparatively poorer, (1.92 per cent) in nitrogen than immature plants (3.49 per cent) and richer in carbohydrates, neither ammonification nor nitrification was possible since bacteria and fungi prefer carbohydrates to proteins as a source of energy (Waksman [24]). The young plants rich in protein and with lesser carbohydrates, ammonify and

nitrify rapidly. Low ammonia content in the beginning indicates immediate nitrification of any ammonia formed. Under field conditions, a slight increase in the nitrate was due to nitrification of the microbial cell substance formed during the earlier part of the decomposition process.

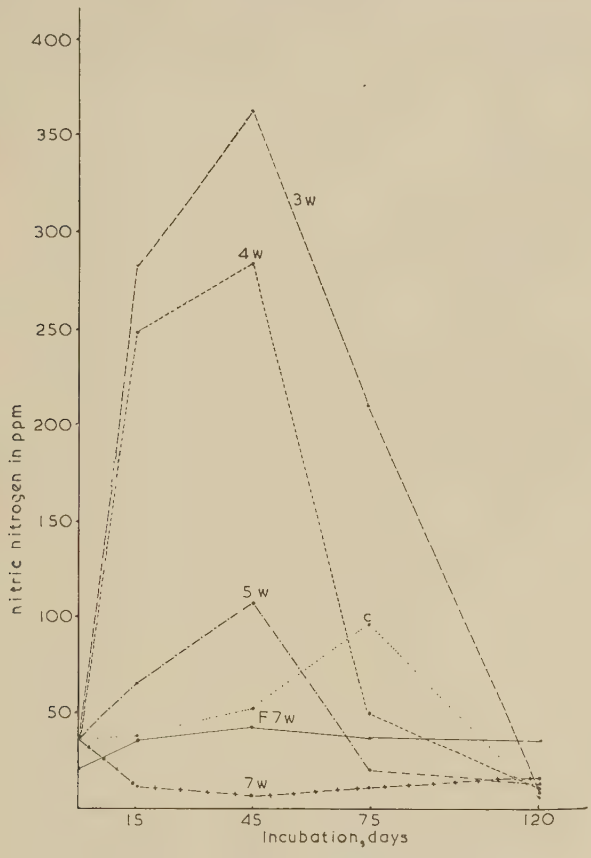

Fig. 5. Changes in nitric-N during decomposition.

A considerable amount of total nitrogen was lost in the immature plants treatments (Fig. 6). About 60 per cent of the total nitrogen was lost in 120 days with immature plants (3 weeks), while only 19 per cent was lost with the mature plants (7 weeks). Even under field conditions, nitrogen was lost with 7-week plants.

Several authors have recorded considerable losses of nitrogen during the decomposition of green plant materials (Daji [7], Ghil-

dyal [11] *etc.*). When readily decomposable, nitrogen-rich organic matter is mixed with the soil, nitrogen is lost as ammonia (Russell [19]), since plant materials of narrow C : N ratio tend to liberate ammonia on decomposition (Acharya [2]). The loss may also be due to denitrification favoured in the presence of an easily oxidizable

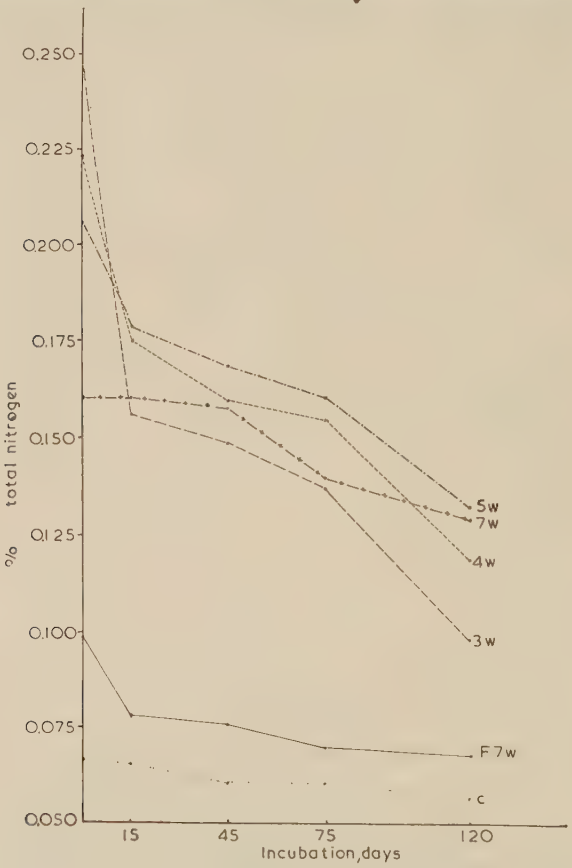

Fig. 6. Total-N content during decomposition.

carbon source at pH 7.2—7.8 (Waksmann [23]). The immature plants which nitrified quickly, suffered heavy losses of nitrogen. These losses, therefore, seem to be related to nitrification. In the process of nitrification, nitrogen may be lost in the gaseous form, due to the formation and decomposition of unstable ammonium nitrite (Dhar *et al.* [9]) or due to the interaction between ammonium and nitrite ions (Wahhab and Fazaluddin [22]).

pH value

A decrease from 7.8 to 7.2 in pH was observed after an incubation period of 15 days with immature plants of 3 and 4 weeks (Fig. 7).

But later the alkalinity increased, showing a temporary influence of decomposing organic matter on the pH in calcareous soils. It is, however, interesting to note that the fungus flora was quite active at pH 7.6 with the 7-week plants.

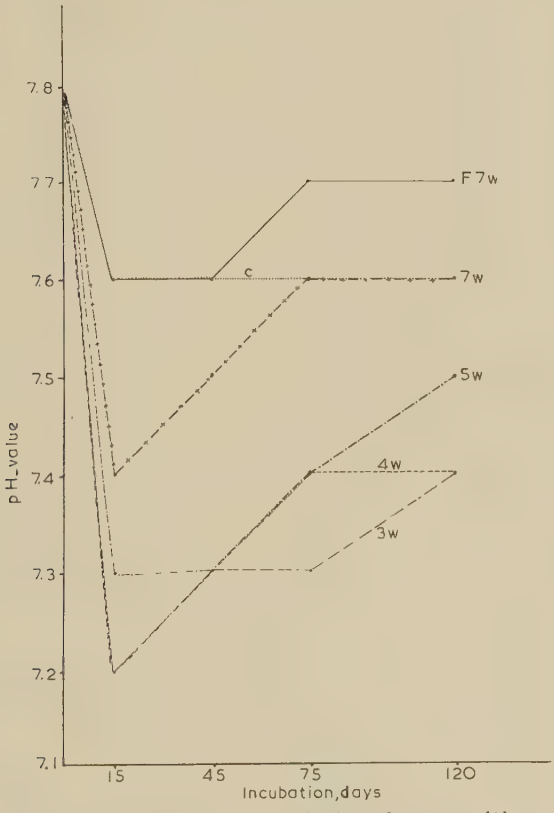

Fig. 7. Variations in pH during decomposition.

Total and water-soluble organic matter

About 60 per cent (Fig. 8) of the organic matter was lost during the first 15 days of decomposition period with immature plants, while with mature plants only 62 per cent was lost in 120 days, and under field conditions, only 21 per cent was lost in the same period.

About $^2/_3$ of the water-soluble organic matter (Fig. 9) in the

immature plant treatments disappeared within a short period of 15 days incubation. With 3-week sann hemp plants about 66 per cent of the water-soluble organic matter was lost after 15 days and about 85 per cent within 120 days. With 7-week mature plants only 51 per cent was lost after 15 days and 79 per cent within 120 days. Under field conditions also the rate of decomposition was slow.

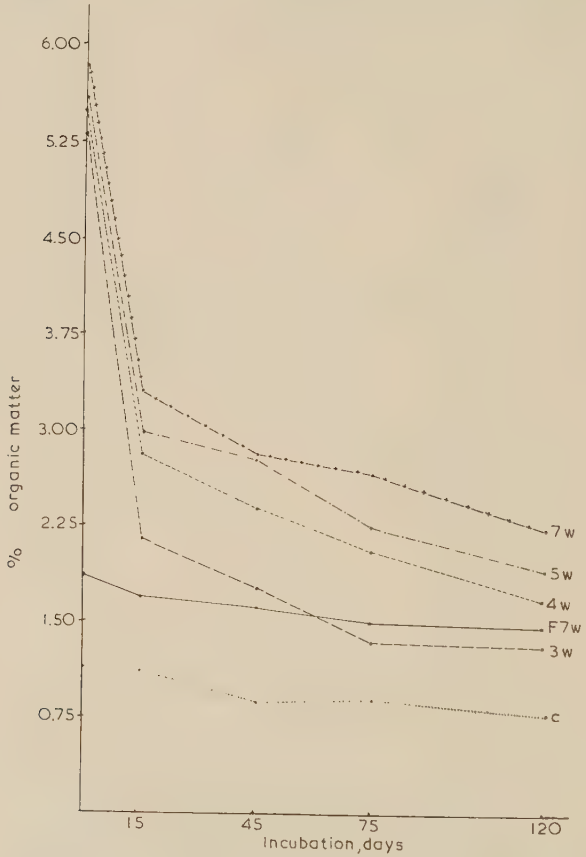

Fig. 8. Decomposition of organic matter

The fall of the content of water-soluble organic matter below that of the control seems to point to the priming action *i.e.* "increase in decomposition of native soil organic matter as a result of microbial stimulation by addition of residues" (Stotzky and Mortensen [21], Broadbent [5], Hallam and Bartholomew [12]).

The disappearance of the water-soluble organic constituents was fairly closely related with the number of bacteria. With younger plants richer in this fraction a greater increase in the number of bacteria was observed. A decrease in the water-soluble organic matter with the maturity of the plants was accompanied by a decrease in the bacterial numbers.

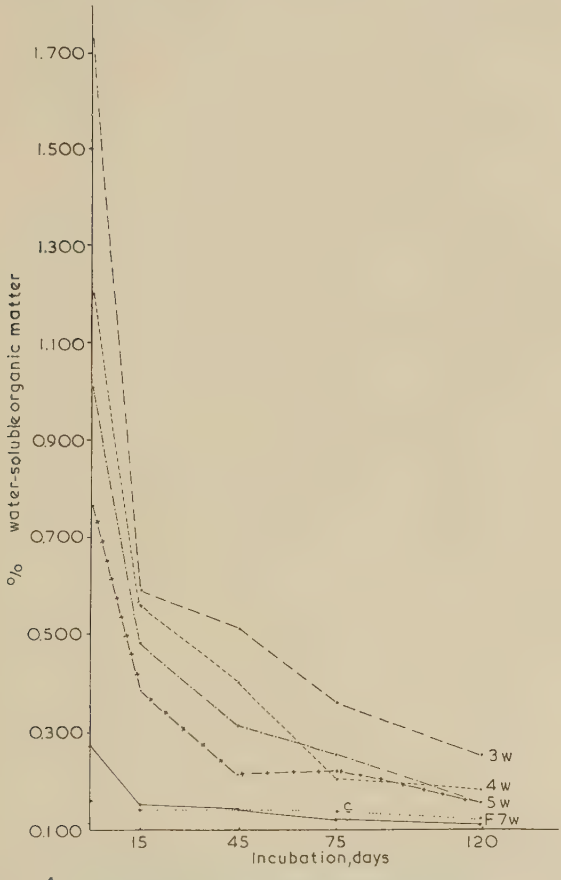

Fig. 9. Decomposition of water-soluble organic matter.

Ether extract

Ether extract includes fatty substances, which are resistant and suffer little loss (Fig. 10). In 3-week plants about 20 per cent of ether extract was lost in 15 days, while 36 per cent was lost in 120 days. In the mature plants only 14 per cent was lost in 15 days and

28 per cent in 120 days. But under field conditions the ether extract fraction decomposed completely and could not be detected after 45 days.

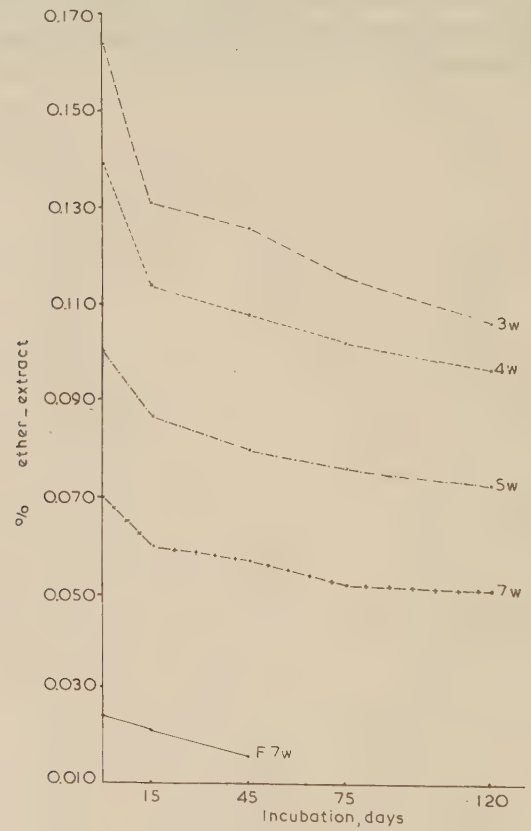

Fig. 10. Decomposition of ether extract.

Pentosans

When 3-week plants of *Crotolaria juncea* were mixed with soils, about 60 per cent of the pentosans (Fig. 11) disappeared within a short period of 15 days. In mature plants only 50 per cent of the pentosans was lost in 15 days and 64 per cent in 120 days. Under field conditions only 53 per cent was lost after 15 days and 62 per cent after 120 days.

Pentosans like water-soluble organic matter also disappeared to a large extent within 15 days showing the major role of bacteria in

the process. The rapidity of the decomposition of pentosans of young plants seems to be due to sufficient available nitrogen for the activity of bacteria. While the decrease in the rate of loss of pentosans during the later stages of decomposition may be partly due to its association with cellulose and lignin (Rege [18]) and partly

Fig. 11. Decomposition of pentosans.

due to the synthesis of microbial polysaccharides resistant to decomposition (Waksman and Tenny [25]). Low bacterial number at this stage may also be responsible for the slow loss of pentosans and it seems that here fungi and actinomycetes do not prefer pentosans to cellulose for their energy requirements.

Celluloses

The cellulose of young plants was lost more quickly than of mature plants (Fig. 12). Three-week plants of *Crotolaria juncea* suffered a loss of about 50 per cent in 15 days and 90 per cent in 120 days, while in 7-week plant treatments 41 per cent cellulose was lost in 15 days and 75 per cent was lost in 120 days. Under field conditions about 50 per cent was lost in 15 days, while after 120 days only 68 per cent was lost.

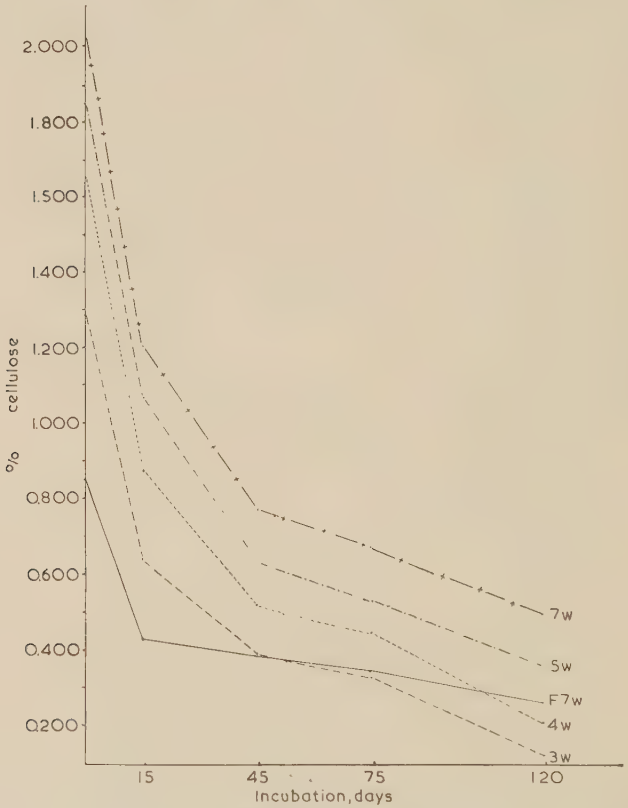

Fig. 12. Decomposition of cellulose.

During earlier stages of decomposition bacteria seem to play a predominant role in utilising the cellulose. About 40 to 50 per cent of the cellulose was lost in all treatments within 15 days when bacterial activity was maximum. The number of fungi and actinomycetes increased as the decomposition of green plants proceeded. This

increase was more where mature plants rich in cellulose were allowed to decompose. Fungi therefore seem to utilize the older tissues better.

There is a close relationship between the carbonaceous substances of the green manure and micro-organisms. Fungi can use as much as 30 to 40 per cent of the carbon of the organic matter decomposed for the synthesis of cell substances (Waksman[23]). Mature plants

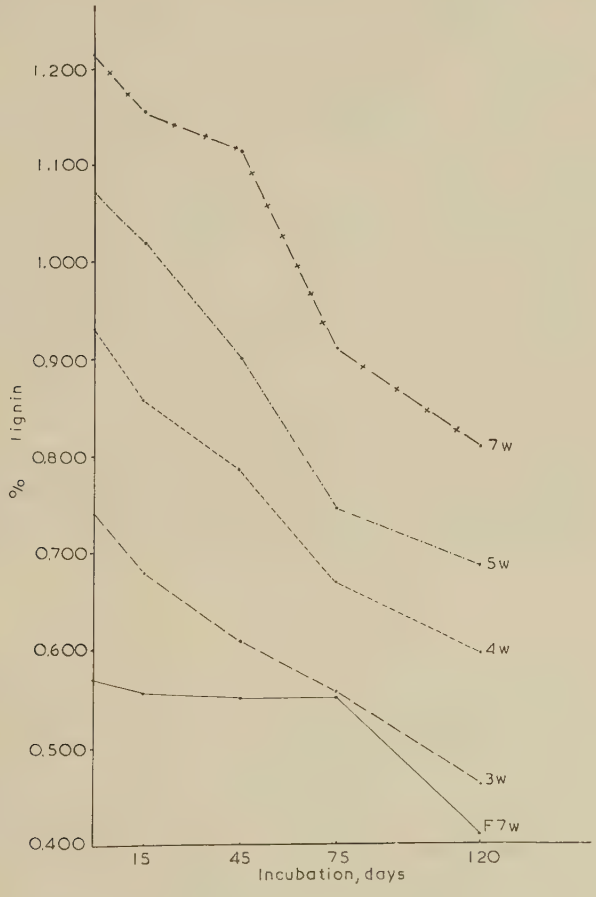

Fig. 13. Decomposition of lignin.

with more cellulose and lignin are richer in carbon and are therefore utilized by fungi and actinomycetes which require more of carbonaceous matter. This explains why the bacterial number was higher with the immature plants and why fungi and actinomycetes were more with mature plants.

The decreased rate of loss of cellulose of mature plants was due to the high content of lignin, which by encrusting the cell wall acts as a barrier to the utilization of celluloses by micro-organisms (Norman [16] and Daji [7]).

Lignin

Lignin was among the most resistant chemical complexes (Fig. 13). As is clear from the figure, about 8 per cent was lost in 15 days in the treatments with young plants and 38 per cent in 120 days, while only 4.9 per cent of lignin of mature plants was lost in 15 days and 33 per cent in 120 days. Under field conditions 28 per cent was lost in the same period.

Though the sann hemp plants were fairly rich in lignin – 14 to 21 per cent – the rate of decomposition of the plant material was not seriously affected. This may be either due to the nature of the lignin or due to sufficient nitrogen available for the decomposition process.

Fungi and actinomycetes seem to play an important role in the decomposition of lignin of sann hemp. This becomes evident from the small losses during early stages of decomposition when the number of fungi and actinomycetes was not high, as compared to other constituents like water-soluble organic matter, pentosans, celluloses, *etc.* But later, with increase in the numbers of fungi and actinomycetes, lignin suffered appreciable losses.

SUMMARY

1. The common Indian green-manure crop *Crotolaria juncea* (sann hemp) was used for studying the biochemical and microbiological changes during its decomposition in a calcareous soil.

2. The composition of sann hemp plants at various stages of growth (3, 4, 5, and 7 weeks) has been reported and the course of decomposition of these plants was followed by measuring the losses undergone by major plant constituents and determining the variations in the number of micro-organisms both under optimum laboratory and field conditions.

3. The bacterial population registered a sudden increase in the beginning and a similar decrease later. With immature plants and during earlier stages of decomposition the bacterial number was high, whereas fungi and actinomycetes were more active with mature plants and during the later stages of decomposition utilizing the complex carbonaceous matter.

4. Proteins of young plants – 3 and 4 weeks old – mineralized rapidly and a considerable amount of total nitrogen was lost, whereas proteins of mature plants were not readily mineralized in which case little nitrogen was lost.

5. During the early periods of decomposition considerable amounts of water-soluble organic matter, pentosans, and cellulose were lost, while lignin, fats and waxes decomposed slowly.

6. The role of bacteria, actinomycetes, and fungi in the decomposition of various plant constituents has been discussed.

7. The decomposition of various plant constituents, nitrogen transformation and microbial activity under field and laboratory conditions has been compared and notable difference has been observed in the nature of the two processes.

ACKNOWLEDGEMENTS

Our thanks are due to Dr. J. G. Shrikhande, Principal and Professor of Chemistry, Government Agricultural College, Kanpur, for his valuable help during the course of investigation.

The junior author is indebted to I.C.A.R., New Delhi, for awarding him a Senior Research Fellowship in Agricultural Chemistry which enabled him to pursue the present problem.

Received July 31, 1958. Revised draft received June 3, 1959

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Plant and Soil XI, no. 4 November 1959

SOME EFFECTS OF THE FLOWERPOT ON PLANT GROWTH

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PART 1 – PHYSICAL PROPERTIES OF FLOWERPOTS

Introduction

Previous workers in this field have found a rapid deterioration of the nutrient status of soil or compost when placed in flowerpots and considered that this was at least partly due to the action of the porous pot wall. In addition, indications were obtained that the chemical nature of the pot wall might well have a direct action on the plants themselves, as much of the root system is found to grow in close proximity to it. Recently, several new materials have been introduced for the manufacture of flowerpots although probably none of these can be said, as yet, to perform efficiently all the functions of the clay pot. In order to be able to assess the suitability of these new materials and throw more light on the effects mentioned above, it was felt that more should be known about the behavior of the ordinary earthenware pot. Accordingly, five types of pots were obtained from various makers in this country and the relevant physical and chemical properties examined on a comparative basis. In conjunction with this, a comparison was made of the growth of plants in the different types of pot under the same external conditions. Thus, the main object of this investigation was to attempt an answer to the practical question, "Does the type of pot used, affect the growth of a plant and if so, why?"

Materials

The five types of pot obtained, were designated A, B, C, D, and E for convenience. Type D and E were machine pressed and the remainder hand made. The C and D types were from the same maker. The pots were obtained in four sizes ranging from three to six inches in diameter and all were new.

Results

The open-pore system of the flowerpot wall has an important bearing on the movement of water and nutrients in the soil which

will be discussed later. A study of the physical properties of the pots was therefore directed towards a better understanding of this porous system. The main features of this part of the investigation are described here.

The total porosity of a pot is that percentage of the volume of the pot wall which is occupied by pores. This was, in effect, determined by subtracting the volume of the solid material itself, (obtained by powdering the pot and measuring the density of the material) from the total volume of the pot wall including the pores. The porosity values obtained for types A, B, C and D were all remarkably close to 28 per cent, while the E type showed a much higher value, namely 39 per cent.

The apparent porosity of a pot is that percentage of the total volume occupied by such pores as are capable of being filled with water. The values were determined simply by immersing pots in water and finding the increase in weight after varying periods. It was found that in each type of pot the weight increased only gradually, corresponding with the slow penetration of water into the pores. After three months immersion, the values for apparent porosity in the C, D and E type of pots had become equal to those for the total porosity, showing that after this time the entire pore system had become filled with water. On the other hand, in the A and B types, the apparent porosity values were still 8 per cent below those for the true porosity and were still slowly increasing. Some of the pores of A and B types were presumably much smaller than in the other types since they were so slow in filling.

The permeability of the pots to air was next determined. Permeability differs from porosity, for, whereas two pots may have the same porosity, one may have much larger (and consequently fewer) pores than the other and will have the larger permeability, since one wide pore offers much less resistance to the passage of air than a number of small pores of the same length and the same total area of cross section. Thus, by determining the permeability to air of the pots walls it is possible to obtain directly an idea of the average pore size in the various types. The values obtained in the various types and sizes are shown in Table 1.

The differences in permeability between the different pot types were very marked. Thus, passing from type A to type E, there was a progressive increase in permeability, which was thought to

TABLE 1

Permeability to air $\left(= \frac{\text{Litres of air passing} \times \text{thickness of wall (cm)}}{\text{Area of pot (sq. m)} \times \text{pressure difference (mm Hg)}} \right)$				
Type	Size of pot			
	3″	3½″	5″	6″
A	0.010	0.012	0.014	0.020
B	0.058	0.057	0.074	0.067
C	0.46	0.23	0.34	0.41
D	0.13	0.22	0.47	0.39
E		1.51	1.72	1.36

correspond to an increase in pore size. The permeability values of all types of pot were, however, so small as to indicate that direct aeration of the plant roots through the wall could not take place to any appreciable extent. This is in agreement with the views of the previous workers.

The permeability to water was measured next; pots were almost filled with water after blocking the drain hole. The tops were covered with tin foil to prevent surface evaporation and the pots were left standing on the laboratory bench. The daily loss in weight due to the water passing through the wall and evaporating on the outside was found. The mean values for each type are given in Table 2.

TABLE 2

Relative evaporating capacity (per cent). (Average loss through pot wall as % of that from a free water surface of same area as pot wall)					
Type.	A	B	C	D	E
	4.4	7.3	50	60	78

Thus, there were very large differences between the behavior of the pot types just as in the case of the permeability to air. As before, the values increased steadily from type A to type E confirming that the pore size increases in the same manner. The ratios of the extreme values (A to E) were not so wide as in the case of the permeability to air; this was to be expected however, on theoretical grounds.

Finally, a purely practical experiment was made to find the daily loss of water through the sides of pots under ordinary greenhouse conditions. Small pots with the drainage hole blocked were immersed in water for 24 hours. They were then filled with compost having a definite moisture content (22 per cent on dry

weight); the tops were covered to prevent surface evaporation and the pots placed on the greenhouse bench. The daily loss of water was found by weighing and made good each day. At the same time the loss from the moist soil surface was found using uncovered glass pots.

For each type of pot it was found that there was a definite daily loss of water, due to water passing from the soil pores into the pores of the pot, then through the pot wall to evaporate on the outside. In the A and B types of pot, the loss from the walls amounted to only about half of that from the upper soil surface; in the C and E types, the loss was about the same from each cause, while in the D type, twice as much water was lost from the walls as from the soil surface. The actual mean daily losses in grams for five inch pots, in cool October weather, are shown in Table 3.

TABLE 3

Daily losses by evaporation from moist compost through the pot wall (grams) (under greenhouse conditions with tops sealed)					
Type. .	A	B	C	D	E
	2.5	4.2	11.3	20.8	10.5

It can be seen that there was an increase in the daily loss of water with increase in pore size (as indicated by the other physical properties) passing from type A to type D. In type E however, the loss had fallen off even though it had been shown that the pores in type E are larger than those in type D. There are also theoretical grounds for expecting the daily loss of moisture from pots filled with moist soil to increase with pore size up to a certain point and then decrease again. This is further supported in practice by the fact that cement pots which have very large pores do not lose water from their walls by evaporation .Thus, it was considered that the water losses through the walls of the D pots were close to the maximum which could be expected for any type of pot.

Thus, to summarise the results of the examination of the physical properties, it may be definitely stated that although the pots from various sources were fairly similar in appearance to one another, they differed a great deal in pore size and in practice by the amount of the daily loss of water through the walls.

PART 2 – THE CHEMICAL PROPERTIES OF POT MATERIAL

Plant nutrients present in pot material

Pots are normally manufactured by firing clay, of a type closely resembling ordinary soil clay, to a temperature between 600° and 900° C. Ordinary soil clay normally contains many elements of nutritional value to plants, the most important of these potassium and phosphorus. If large quantities of these elements are liberated by the action of heat during the manufacture of pots, and were retained in the finished pot, the available plant food so formed might considerably benefit a plant grown in a new pot. An investigation was therefore made of the nutrients present in new pot material.

The results of a preliminary examination of matter soluble in constant-boiling hydrochloric acid (Type-A material) (1 hour) are shown in Table 4.

TABLE 4

Type-A Pot material – Portion soluble in constant-boiling hydrochloric acid	
Water (lost below 600° C) . .	0.64 per cent
Insoluble matter	86.09
Silica	0.39
Phosphorus pentoxide	0.15
Ferric oxide	4.62
Alumina	3.02
Potash.	0.67
Calcium oxide	1.99
Magnesium oxide	1.38
Sulphate.	0.88
	99.83

Thus, the pot material contained all the major plant nutrients found in soils, with the exception of the nitrogenous compounds, which, even if present initially, were presumably lost during the firing. The greater part of the material, (probably consisting of silica, alumina and ferric oxide) was insoluble.

It cannot be assumed that the soluble nutrients shown in Table 4 are entirely available to plants, but the data served to locate useful nutrients, less inert than the bulk of the pot material and therefore possibly utilisable by plants given time and suitable conditions.

As a measure of the proportion of these nutrients more or less easily available (*i.e.* during one season) to plants, the generally

accepted citric-acid extraction test was applied to the pot material. Typical results are shown below (Table 5).

TABLE 5

Solubility of nutrients from pot material in 1 per cent citric acid (parts per million)					
Type of pot	A	B	C	D	E
Phosphate (P_2O_5)	480	290	195	175	170
Potassium	254	247	540	534	425
Calcium	5350	5200	2900	2750	7800

These results were similar in relative order to those obtained with other solvents, commonly used to measure "available" nutrients in soil. On the whole, the relative amounts were rather similar to those in ordinary soils.

To reinforce the evidence of the chemical tests for availability of nutrients in the pot material, a direct test of availability was made by growing plants in ground-up pot material instead of soil. The type-A pot material, to which ammonium nitrate was added, was used for this experiment, the test plants being mustard and radish. At the same time, seeds were sown in an ordinary potting soil to act as controls. The seeds in the pot material germinated and grew although more slowly than those in soil. At the end of three weeks the plants in the soil were sampled for analysis. The plants in the pot material took six weeks to reach the same size. The average percentage composition of the plants is shown in Table 6.

TABLE 6

Direct availability of nutrients from pot material, to growing plants. Plant analysis (%)					
Nutrient	N	P_2O_5	K	Ca	Mg
Mustard in pot material	5.43	0.115	0.54	2.57	1.20
Mustard in soil	4.24	1.22	1.36	2.37	0.26
Radish in pot material	6.09	0.147	0.47	3.16	1.42
Radish in soil	4.16	1.27	1.29	2.93	0.34

The uptake of phosphorus by the plants in the pot material was very much smaller (10%) than that by the plants grown in soil. Since the chemical tests showed that this A-type pot material was the richest in phosphate, it was quite clear that the phosphorus

in pots is of little benefit to plants. The potash uptake was slightly greater but was still very small in view of the large demand of the plant for this element. The pot material appeared able to supply all the calcium needed by the plant under the conditions of this experiment, while the plants grown in pot material actually contained four times as much magnesium as the control plants; the reason for this large uptake is not yet known.

Absorption of nutrients by pot material

It has already been shown that under normal conditions there is a constant stream of water from the soil through the wall of the pot to the outer surface, where the water evaporates. Dissolved in this water are soluble plant nutrients from the soil, the dilute solution being known as the soil solution. There is thus a constant tendency for these nutrients to be carried into the walls of the pot. Once inside the pores of the wall, three possibilities may arise, the nutrients may crystallise on the outside of the pot: this often happens on old pots on which a white incrustation may be seen. If the pot material or any constituent of it is reactive, the nutrients may combine with it chemically and thus be removed from solution. Finally, if neither of the above two possibilities occur in the case of a given nutrient, it may just remain in solution within the pores of the pot, the solution gradually becoming more concentrated. In this latter case, the possibility of the nutrient being able to pass back into the soil and being taken up by the plant arises.

New pots were alkaline compared with the majority of soils. In addition they showed some resistance to the lowering of pH on the addition of acid. Thus, even after containing an acid soil for two months, the pot material still remained alkaline (Table 7).

TABLE 7

pH of pot material and effect of acid soil compared with original clay

Type of pot.	A	B	C	D	E
pH of new pots	8.67	8.66	9.36	9.59	8.80
pH of parent clay	7.85	7.70	8.12		7.96
pH of pots filled with soil for 2 months. . .	7.28	7.16	7.55	7.75	

Neither potash nor nitrate was absorbed by pot material. In the case of phosphate however, a marked absorption was noted, and

this was accompanied by a corresponding fall in the amount of soluble calcium in the pot material. Presumably, precipitation of calcium phosphate occurs under the alkaline conditions in the pot pores, and therefore phosphate entering the pores of a pot may be rendered wholly or partly unavailable to plants by this means. Previous work carried out in this Department showed a steady diminution in the soluble-phosphate content of potting soil after placing in pots. Thus the importance of a generous proportion of phosphate in potting soils already well known for other reasons, receives additional emphasis. Finally, the various soluble constituents of the walls of flowerpots which had contained growing plants, were analysed and the values obtained compared with those for unused pots. It was found that the porous walls showed a tendency to accumulate soluble nutrients from the soil, although in some cases the interpretations were obscured by the rather variable amount of soluble matter in new pots. In addition, indications were obtained that the nutrients except phosphate, were able to diffuse back from the pores of the pot into the soil. Thus, in the case of nitrate, it was found that there was less of this in the pot wall after two months growth than there had been at the end of one month. Presumably, some had diffused back into the soil and been absorbed by the plant, as shown in Table 8.

TABLE 8

Nitrate content (ppm) of pot material during plant growth				
Type of pot	A	B	C	D
Nitrate in new pot	—	—	—	—
Nitrate after one month	29	22	45	61
Nitrate after two months	16	4.7	4.5	22

PART 3 – A COMPARISON OF THE GROWTH OF PLANTS IN VARIOUS POT TYPES

An important feature characterising the various types of pot was the large variation in the permeability. In practice, this manifested itself in the great differences in the daily loss of water by evaporation from the outside of the pot wall. The effects of differences in permeability and evaporation rates of different types of pots on the growth of plants was examined.

Flowerpots of types A, B, C and D each in sizes of 3½, 5 and 6 inches were used together with dark brown glass pots (G), representing complete impermeability. Mustard was used as a test plant because of its hardiness and ability to grow in a limited quantity of soil.

Throughout this experiment, one plant was grown per 250 grams of soil, there being respectively 1, 4 and 8 plants in each of the three sizes of pot used. The soil used for this experiment was a silty clay loam of good nutrient status, to which was added about a quarter of its bulk of shredded peat. Care was taken that all plants were treated in exactly the same way except for the effects actually due to the differences in pot type. Thus, the mixture of soil and peat was very thoroughly mixed in the dry state before use, and the position of the pots on the greenhouse bench was frequently moved around so that differences in plants, lighting etc., were minimised. Fallow pots equal in number to the planted pots were included in the experiment and were arranged so that each planted pot was immediately surrounded by four fallow ones to minimise shading effects. The seed was sown a week after filling the pots with moist soil, and watering was carried out as often as seemed necessary, usually twice daily during the hot weather.

The soil in the glass pots was always noticeably more moist than that in the other types. The D type pots were driest and the C pots almost as dry. Thus, the moisture content of the soil decreased with increasing permeability of the pots. This state of affairs persisted even though the drier pots were always given much more water, it being endeavoured to treat each pot according to the requirements of the plants in it.

Extraction and analysis of the soil solution (the actual water with its dissolved nutrients *etc.*) was made on three occasions during the growth period, from both fallow and planted pots. On the whole, the concentration of nutrients in the soil solution decreased with time, the effect being more pronounced in pots having greater permeability, owing to the increased rate of passage of soil solution into the pores of the pot wall. Another important fact, shown by the soil solution data, was that in the planted pots, as soon as the plants had produced sufficient root systems, the concentration of nitrate in the soil solution dropped, virtually to zero, despite the high nitrogen status of this soil. Presumably, after this stage had been reached, the plants depended entirely on the production of nitrate from the nitrogenous organic matter by soil micro-organisms (nitrification). In the fallow pots, at the time of the first sampling, the concentration of nitrate was least in the most permeable pots (D), as was the case with the other elements. This was due to the

high rate of passage of the soil solution into the pot wall. At the end of the growth period however, exactly the reverse was the case, the concentration of nitrate being greatest in the more permeable pots. This reversal indicates that nitrification took place at the greatest rate in the most permeable pots, *i.e.* those permitting the greatest aeration of the soil. This aeration seems to take place not directly through the pot wall, but indirectly due to air entering the soil at the free upper surface to take the place of water passing outwards through the pot wall. The various features of the nitrogen status of the soil solution are illustrated by the data given in Table 9.

TABLE 9

Nitrate in soil solution from 6-in. pots. (ppm of dry soil)						
Type	Planted			Fallow		
	1st sampling	2nd sampling	3rd sampling	1st sampling	2nd sampling	3rd sampling
G	21.0	0.5	0.7	58	12	4.1
A	0.6	0.2	0.9	39	28	4.7
B	0.2	0.3	0.6	40	21	6.3
C	0.3	0.2	0.5	25	12	19.0
D	7.8	1.5	0.9	19	25	18.0

The seeds germinated within two days and growth took place rapidly. At first the plants in the low-permeability A- and B-type pots made the best growth, those in the C- and G-type pots were practically as good, while the plants in the D-type pots were noticeably poorer. This was attributed to a shortage of moisture due to the rapid drying out of the soil in the very premeable pots, in spite of copious watering twice daily.

At the end of four weeks, when the flower buds began to form, a noticeable improvement was seen in the plants in the C and D types of pot, relative to other types. They remained green while the others became yellow and sickly in appearance. In this way, by the end of the growth period, the C plants had become best of all, while the D plants, still somewhat hampered by the lack of water, were only slightly better than those in the glass pots.

At the end of eight weeks growth, when the seeds were forming, the plants were cut off at soil level, dried, weighed, and analysed chemically. The yield data Table 10 confirmed the visual observations of the grown plants.

TABLE 10

Mean dry weight of plants in milligrams (at 8 weeks)					
Pot type	A	B	C	D	G
5-inch pot	393	409	502	365	323
6-inch pot	509	471	584	357	333

Thus, in each size of pot, the plants in the medium-permeability C pots were largest, those in the A- and B types of lower permeability next, followed by the plants in the D pots of high permeability, while the impermeable glass pots showed the lowest yields.

The figures obtained from analysis of the plants Table 11 showed a striking increase in the uptake of nitrogen with increasing permeability of the pots.

TABLE 11

Uptake of nitrogen by plants (milligrams per plant)					
Type of pot.	G	A	B	C	D
5-inch pot	3.23	4.60	5.49	6.09	6.50
6-inch pot	3.90	5.72	5.85	7.89	7.35

This confirmed the data from analysis of the soil solution in showing that increased nitrification had taken place in the more permeable pots, resulting from increased soil aeration.

It has been frequently noticed that nitrification in the soil is often accompanied by an increase in available potash due to the action of the same soil organisms. An increase in the uptake of potash by the plants, corresponding to that for nitrogen was also noted in this experiment (Table 12).

TABLE 12

Uptake of potash by plants (milligrams per plant)					
Type of pot.	G	A	B	C	D
5-inch pot	5.1	8.2	7.7	11.2	9.7
6-inch pot	5.4	10.5	10.2	10.3	10.2

Examination of the roots of the plants showed that those in the C and D types were white and healthy looking, with many root hairs. The roots in the A and B pots were inferior to these and showed less branching, while those in the glass pots were discoloured and unhealthy in appearance.

SUMMARY

This investigation has thus shown that while pots from various sources differ but little in appearance, there are considerable differences in the permeability of the walls, due fundamentally to variations in the pore size. The characteristic features of porous pots in that there is a continuous current of soil solution outwards from the soil through the pot wall, the rate of this current increasing with permeability. Air to replace this water enters the soil at its upper surface, thus, the extent to which the soil is aerated is indirectly determined by the permeability of the pot. This increased aeration has been shown to lead to an increased rate of nitrification in the soil, together with an increase in the available potash, resulting in greater plant yields. Good aeration of the soil also had a beneficial effect on root development.

Thus, in general, high permeability is advantageous in pots, unless it is so great as to lead to such rapid drying of the soil that the plant suffers drought effects. Such detrimental effects are particularly likely to occur under extreme conditions of high temperature and low humidity.

Another important point, emphasized in this study is the large demand of the pot plant for nitrogen in comparison with the small amount of soil explorable by its roots. Addition of nitrogen is therefore desirable in any case, while for impermeable pots in particular, it is advisable that such nitrogen should be directly assimilable rather than in an organic form.

Received February 17, 1959

Plant and Soil XI, no. 4 November 1959

FACTORS INFLUENCING THE ISOTOPICALLY EXCHANGEABLE PHOSPHATE IN SOILS

PART I. THE EFFECT OF LOW CONCENTRATIONS OF ORGANIC ANIONS

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INTRODUCTION

The rate of isotopic exchange of phosphate ions between the soil solution and the soil has been used to divide the total labile phosphorus into 'rapid' and 'slow' fractions by an examination of the curve giving the decrease in P^{32} activity of the solution with time (Talibudeen [10]). Earlier work on soil suspensions by other investigators referred to previously, had indicated the possibility of such a sub-division but no quantitative work had been described. Amer *et al.* [1] examined the rates of removal of phosphate ions from soils by anion-exchange resins and found that the data are qualitatively similar to those on the isotopic exchange of phosphate ions between the soil and the soil solution. They showed that the observations on the resin–soil experiments "could be fitted by assuming that there were three simultaneous first-order reactions", and state that "the sources of P involved in the three hypothetical reactions are unknown". The reactions are considered as "behavioural patterns characterized by a quantity and a rate....". Although data on the rate of P^{32} equilibration are given for two soils, detailed analyses were not attempted. Seatz [8] also suggested the existence of three separate reactions in the isotopic exchange of phosphate ions in soil systems without giving any detailed analyses.

In non-soil systems, Govaert *et al* [4] studied the isotopic exchange of Ca^{45} and P^{32} in suspensions of "tricalcium phosphate". With phosphate ions, they observed three separate kinetic reactions with half-lives of exchange 16.7 minutes, 9 hours, and 222 days. Neuman *et al.* [11] obtained similar results with apatite crystals of bone suspended in phosphate buffers. Isotopic exchange of C^{45}-labelled CO with CO adsorbed on iron catalysts

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was used by Eischens [3] to study the surface heterogeneity of the precipitated iron; again three reactions were detected.

In our work the results for the isotopic exchange of phosphate ions obtained with two soils were analyzed by methods similar to those of Eischens [3] and Amer *et al.* [1]. To make this possible the reactions are assumed to be confined to the surface of the solid, and a high speed of agitation of the system is maintained. In this and succeeding papers, we shall investigate the nature of these fractions by a study of their interaction with external conditions. Such measurements and their treatment may be equally valuable in investigating the surface activity of other nutrient ions in the soil, *e.g.* potassium, ammonium, nitrate, calcium and micronutrient elements, if suitable isotopes are available.

When isotopically exchangeable (or total labile) phosphate is determined in soil suspensions containing decreasing concentrations of citrate ions (Talibudeen [9]), a limiting citrate concentration of 0.001 M is observed below which the total labile phosphate does not alter as the citrate concentration falls to 0.0001 M. However, the phosphate displaced into solution decreases continuously with decreasing citrate concentrations. The behaviour of different soils under such conditions in regard to the total labile phosphate and the phosphate displaced into solution is discussed elsewhere (Talibudeen [9]). In the following sections, the distribution of phosphate ions in the total labile phosphorus determined in the absence of organic anions is compared with the distribution in the presence of this limiting concentration of citrate ions and also in the presence of the same concentration of the non-chelating anion of diethyl barbituric acid.

KINETICS OF ISOTOPIC EXCHANGE

McKay [6] derived a simple exponential law to predict the course of the isotopic exchange process in a homogeneous stable system. This can be adapted for soil systems in the following way. If R is the specific rate of exchange of the labelled ion between the phosphate ions in the soil P_s and those in the soil solution P_{ss}, then the fractional exchange F changes with time according to the equation:

$$\ln(1 - F) = -\frac{P_e}{P_s . P_{ss}} . Rt \qquad (1)$$

where P_e is the total labile phosphate in the system and in equal to $(P_s + P_{ss})$ and the fractional exchange F is the ratio of the specific activity at time t in the soil to the asymptotic value. When the labelled ions exchange at two or more different rates because more than one exchanging component occur in the solid, the composite curve can be resolved into its components graphically.

The experimentally determined values of the specific activities of the solution at suitable time intervals are converted to fractional exchange values in the soil F which is given by the equation:

$$F = \frac{\text{Change in specific activity after time } t}{\text{Total change in specific activity value}}$$

METHODS AND MATERIALS

The two soils from Rothamsted Experimental Plots used in this study have been described in detail elesewhere (Talibudeen [10]), essential data are summarized in Table 1.

TABLE 1

Description of soils from Rothamsted Experiments

Site	pH in 0.02 *M* KCl	% $CaCO_3$	% Clay content	Total phosphorus, mg per 100 g soil
Soil A Highfield	6.9 to 7.1	<0.1	20.3	91
Soil B Exhaustion land, superphosphate plot. .	7.5 to 7.7	1.5	19.3	68

The solutions used were made up from analytical grade reagents of ammonia, potassium chloride, citric acid and diethyl barbituric acid. The pH of the solutions of the organic anions were adjusted to those of the soils.

The solutions used were

a. 0.02 *M* KCl

b. 0.02 *M* KCl + 0.001 *M* Citrate (NH_4 form)

c. 0.02 *M* KCl + 0.001 *M* diethyl barbiturate (Na + H form)

(The diethyl barbiturate ion is here-after referred to as the DEB anion). The solution of 0.02 *M* KCl was used as a reference solution to avoid any effects from electrolytes dissolved from the soil and from the different cations in the organic buffer.

To keep the phosphorus concentration as constant as possible during the isotopic-exchange experiment, preliminary runs were made to determine the rate at which 0.5 g of soil came to equilibrium with 100 ml of the different extracting solutions. After 20 to 24 hours continuous shaking at 150 to 200 oscillations per minute, the systems containing organic anions showed a small increase during the course of the isotopic-exchange experiments (ranging from 150 to 250 hours), whereas those without organic anions showed a larger change after the initial rapid rise in the first 24 hours.

Because of this difference, the procedures adopted after labelling with carrier-free radioactive phosphorus were varied. Labelling was done after the initial rapid rise in the phosphate in solution was over. The amount of carrier-free P^{32} was assayed by adding it to control flasks containing the extracting solution together with the levels of P expected in the equilibrium solution. Levels of carrier-P ranging from 6×10^{-7} *M* to 1×10^{-5} *M* were used without showing any measurable change in radioactivity over 7 to 10 days. A half ml of chloroform was added to all flasks to prevent the growth of microflora; this was renewed whenever it appeared to have evaporated. All experiments were at 25° C. The time intervals for sampling were chosen so that more than half the total number of samplings were done in the first 48 hours. Samples were centrifuged at 16000 rpm in an angle head for 5 minutes, temperature control being either between $\pm$ 1° or between 0° and 5° C. Where the larger temperature changes were encountered, the bulk of the soil was removed by sedimentation under gravity or pre-centrifuged at lower speeds (4000–5000 rpm).

The alternative procedures for studying isotopic exchange were as follows.

(A) With organic ions: Three flasks were set out initially and sampled at suitable times by removing approximately 5-ml portions of the supernatant solution. This was centrifuged and the clear supernatant liquid sampled in duplicate with a 0.5-ml micropipette. The P^{32}-content of this 0.5 ml aliquot was estimated as before (Talibudeen[10], Procedure A). The suspension remaining in the centrifuge tube was returned to the reaction flask. Three to four successive samplings were made from each flask. Each flask was centrifuged for the final sampling and analysed for its pH, total phosphorus and P^{32}-content as before.

(B) Without organic ions: One to two μC of P^{32} were added to each of 10 to 12 flasks set out initially. These flasks were removed from the thermostatic water bath at suitable time intervals consecutively and their contents were centrifuged. The centrifugate was analysed for its pH, total phosphorus and P^{32}-content by methods referred to in a previous paper (Talibudeen[10], Procedure B).

RESULTS

All the radioactivity counts were used after correction for decay, lost counts, and also for slight day-to-day variation in the counting apparatus, by referring them to the same P^{32}-standards.

The results obtained with suspensions without organic anions (Procedure B) were converted to specific activities. Radioactivity data from Procedure A in the presence of organic anions were used without further computation because it was considered that the slight increase in phosphorus content in solution over the period

of the isotopic exchange would not affect the rate of isotopic exchange measurably.

The asymptotic value of the specific activity or radioactivity, with respect to time was obtained by repeated measurements at extended time intervals until results remained constant within experimental error.

The expression $1 - F$ was then calculated from the specific activity or radioactivity f_t at time t and the asymptotic value f_∞ by the expression

$$1 - F = \frac{f_t - f_\infty}{1 - f_\infty} \tag{2}$$

The total labile phosphorus in the soil P_s was obtained from the expression

$$P_s = \left(\frac{1}{f_\infty} - 1\right) . P_{ss} \tag{3}$$

where P_{ss} is the amount of phosphate in solution.

The semi-logarithmic plot of $\ln(1 - F)$ against time t, was analyzed graphically to give the amounts of phosphorus P_0, P_1, P_2, and P_3 associated with the 'instantaneous', 'rapid', 'medium' and 'slow' exchanging phosphate fractions in the soil such that

$$P_s = P_0 + P_1 + P_2 + P_3 \tag{4}$$

The total labile phosphate in the system is given by

$$P_e = P_s + P_{ss} \tag{5}$$

From the graphical analysis the half-lives of exchange ${}^1t_{\frac{1}{2}}$, ${}^2t_{\frac{1}{2}}$ and ${}^3t_{\frac{1}{2}}$ for the 'rapid', 'medium' and 'slow' fractions could be read off which made it possible to calculate the specific rates of exchange of phosphate ions between those in solution and those in the various fractions by the expression.

$$R_x = \frac{0.693}{{}^x t_{\frac{1}{2}}} \cdot \frac{P_x . P_{ss}}{P_x + P_{ss}} \tag{6}$$

where x refers to the sub-numerals 1, 2 and 3 pertaining to the 'rapid', 'medium' and 'slow' fractions.

Table 2 shows the variation of the total labile phosphate due to the presence of 0.001 M concentrations of organic anions and the effect of the latter on the distribution of the total labile phosphate

TABLE 2

Effect of organic anions on the isotopically exchangeable phosphorus and its fractions
All figures in mg P per 100 g soil per 20 litres solution

	P in solution at time of labelling with P^{32} P_{ss}	P in solid, P_s					Tota labile P_e
		Instantaneous P_0	Rapid P_1	Medium P_2	Slow P_3	Total in soil $P_0 + P_1 + P_2 + P_3$	
Soil A							
0.02 *M* KCl	0.80	1.69	0.23	0.42	8.16	10.52	11.3
+ 0.001 *M* citrate . .	6.58	3.01	1.08	1.69	3.86	9.64	16.3
+ 0.001 *M* barbiturate	2.25	0.78	0.59	0.77	4.68	6.81	9.0
Soil B							
0.02 *M* KCl	1.65	1.41	0.28	0.43	7.74	9.86	11.5
+ 0.001 *M* citrate . .	3.66	1.46		0.32	4.01	5.79	9.4
+ 0.001 *M* barbiturate	1.04	1.87		0.59	3.64	6.09	7.1

into its components, the 'instantaneous', 'rapid', 'medium' and 'slow' fractions. In the calcareous soil, the total phosphate exchanging isotopically in the system is decreased by the presence of these low concentrations of organic anions despite an increase in the phosphate desorbed into solution by the citrate ions, whereas the DEB anion increases P_{ss} only for the non-calcareous soil A. The decrease in the phosphate left in the soil after extraction by the organic anions is reflected in a considerable decrease in the amount of the 'slow' fraction P_3. It is also noticeable that the 'instantaneous' and 'rapid'

TABLE 3

Effect of organic anions on the half-life and specific rate of exchange of phosphate ions

	Half-life of exchange (in hours)			Rate of exchange (in mg P per 100 g soil per hour)		
	Rapid	Medium	Slow	Rapid	Medium	Slow
Soil A						
0.02 *M* KCl	1.6	8.6	40.0	0.24 *	0.022	0.013
+ 0.001 *M* citrate	0.5	4.4	38.3	1.21	0.21	0.044
+ 0.001 *M* barbiturate . .	0.4	2.5	25.8	0.44	0.23	0,04
Soil B						
0.02 *M* KCl	0.5	3.4	46.1	1.16 *	0.070	0.020
+ 0.001 *M* citrate	small	2.4	36.2	fast	0.086	0.037
+ 0.001 *M* barbiturate . .	0.3	1.8	27.2	1.49	0.14	0.02

* The 'rapid' rate is calculated approximately since a large part of the rapid component appears to exchange instantaneously.

fractions are clearly distinguishable from each other in *all* exchanges with the non-calcareous soil. With the calcareous soil in the presence of organic anions, however, only one rapidly exchanging fraction is observed.

Table 3 gives the half-lives of exchange and the specific rates of exchange for each fraction and shows that the half-lives of exchange for all fractions in both soils follow the order

no organic anion > citrate > barbiturate

except the 'rapid' fraction in soil B. By contrast, the specific rates of exchange for each fraction are in the order

citrate > barbiturate > no organic ion.

except the 'medium' fraction in both soils.

DISCUSSION

The constancy of the total labile phosphate in a soil at low concentrations of an organic anion like citrate (which strongly chelates alkaline earth cations and cations of iron and aluminium) suggests that this total labile phosphate is a characteristic quantity of the soil related to the physical micro-distribution of phosphates within it. The large and progressive increase in the total labile phosphate because of high citrate concentrations can be ascribed to the dissolution of crystalline phosphates or phosphate ions chemically bound to sesquioxidic or calcareous compounds. With low citrate concentrations, there is no dissolution of a phosphate phase in the calcareous soil, because the total labile phosphate decreases when citrate ions are added; the total labile phosphate in the noncalcareous soil, however, increases in the presence of citrate ions suggesting the dissolution of precipitated phosphates. The phosphate concentration in solution in both soils however is increased over that in the absence of citrate anions due mainly to a decrease in the activity of cations, responsible for phosphate 'retention' in the intramicellar solution, by chelation with citrate ions.

A second-order effect responsible for the increase in the phosphate in solution is an anion-exchange process between the adsorbed phosphate ions and the organic anion. The trivalent form of citrate ions is 80 and 93 per cent of the total citrate concentration at pH values of 7.0 and 7.5 respectively, whereas the corresponding values for the monovalent form DEB are only 29 and 56 per cent respective-

ly. Thus the citrate ion would be much more active in an anion-exchange process at those pH values. The specificity of the adsorbing surface plays an important role in deciding whether anion exchange will occur *e.g.* for soil A, DEB anions increase the phosphate in solution, whereas for the slightly calcareous soil B, they decrease it below the value obtained without of organic anions.

Data concerning the two organic anions, relevant to this study, are given in Table 4. These anions are very similar in their size, and their range of buffering overlaps considerably but their chelating power, at least for calcium ions, is vastly different.

TABLE 4

Data on organic anions				
Anion	Molecular weight	Molecular volume (X-ray)	Dissociation pK_0 at 25° C	Chelation Log K_{Ca}
Citric acid (monohydrate)	210	284 Å^3	6.4 (3rd dissociation constant)	3.15 (Concentration range .0002—.004 *M*)
Diethyl barbituric acid	184	248 Å^3	7.4	(0.66) (Concentration range .016—.15 *M*)

Note; X-ray data are taken from References [2] and [6], dissociation and chelation data from Reference [8].

The effect of organic anions on the labile phosphate left in the soil is to decrease it with the non-chelating monovalent DEB ion to within 62 and 65 per cent of its value in the absence of organic ions, and between 60 and 85 per cent of the original value for the strongly chelating polyvalent citrate ion. As will be shown later, this decrease undoubtedly occurs because the larger organic ions with an average diameter of more than 8 Å block the release of adsorbed phosphate ions (average diameter 4.5 Å) from micropores. The effect of organic anions on the distribution within the total labile phosphate is primarily to decrease the 'slow' fraction. With soil A in citrate solution, over half of this decrease is offset by increases in the phosphate in solution and the 'medium' fraction, and to a smaller extent in the 'instantaneous' and 'rapid' fractions. With the DEB anion, however, the 'instantaneous' fraction is also

decreased whereas only small increases are observed in the remaining fractions. The 'slow' fraction must thus mainly represent adsorbed phosphate buried in micro-pores in the soil of diameter between 5 and 10 Å approximately. As citrate ions can decrease the activity of phosphate-retaining cations in the intramicellar solution, they can desorb more phosphate from these micropores before 'blocking' occurs. With the non-chelating DEB anion these phosphate ions are almost completely locked in and become non-exchangeable isotopically without permitting the movement of PO_4-groups to more easily exchanging sites; the sharp drop in the 'instantaneous' fraction appears to be only partly offset by an increase in the phosphate in solution, suggesting that the DEB anion is able to partly cover PO_4-groups on 'instantaneously exchanging' sites and prevent them from exchanging isotopically.

With the calcareous soil B, the decrease in the total labile phosphate by the addition of organic anions is less pronounced. This is reflected, as in soil A, in the change in the slow fraction P_3, offset by a large increase in the phosphate in solution in the presence of citrate ions. However, although the DEB anion affects the 'medium' and 'slow' fractions P_2 and P_3 as in the non-calcareous soil, it depresses the phosphate in solution significantly and depresses the rate of exchange of the 'instantaneous' fraction so as to make it indistinguishable from the 'rapid' fraction. It is of some interest that, (*i*) despite this internal re-distribution due to the DEB anion, the sum total of $(P_{ss} + P_0 + P_1)$ is the same (2.9 mg per 100 g soil) in the absence and the presence of the DEB anion; (*ii*) the citrate ion mobilises some of the 'slow' fraction to the more readily exchangeable sites in P_0, P_1 and P_2 as well as to the phosphate in solution in soil A, whereas in the calcareous soil B the phosphate moves from the 'slow' fraction directly to the soil solution without affecting the more rapidly exchanging fractions, and (*iii*) whereas citrate invariably increases the phosphate in solution, the DEB anion actually decreases it with the calcareous soil B as noted earlier.

Thus, a comparison of the effects of the two organic anions on the residual phosphate in the soils shows that Soils A and B behave differently. In the $CaCO_3$-free soil, nearly 50 per cent more phosphate is labile in the solid with citrate ions as with DEB ions, whereas in the calcareous soil the effect of the two ions differs much less. This could be attributed to the lower lability of phosphate ions

adsorbed on the calcareous material in soil B in the presence of organic anions and perhaps to the specific effects of the DEB anion on the phosphate retaining surfaces in the two soils. This effect is more clearly brought out when the detailed analyses of the rate-curves is considered (Table 3).

The effect of both organic anions is to greatly decrease the half-lives of exchange for all fractions in both soils. However, except the 'rapid' fraction in soil B, these half-periods are always less for the DEB anion than for citrate. This apparent inconsistency is corrected when the specific rates of exchange are worked out which take into account the partition ratio of the total labile phosphate between the phosphate fraction in the solid and the phosphate in solution. The specific rates of exchange in soil A follow the order

$$\text{citrate} \geqq \text{barbiturate} \gg \text{no organic anion}$$

for the 'rapid' and 'medium' fractions; for the 'slow' fraction the same order is maintained but the effect of the organic anions is small. For the calcareous soil B, however, the order observed for the rapid reaction only is

$$\text{citrate} \gg \text{barbiturate} > \text{no organic anion.}$$

For the 'slow' fraction, the order is

$$\text{citrate} \geqq \text{barbiturate} \geqq \text{no organic ion.}$$

It is also observed that the presence of organic anions does not affect the specific rates of exchange so much in soil B as in soil A except for the 'rapid' fraction in the calcareous soil which is only a very small part of the total labile phosphate. This confirms the earlier suggestion that the labile phosphate in the calcareous soil is less mobile than that in soil A.

There is some evidence in the literature that phosphate groups combined with organic molecules can exchange isotopically with orthophosphate ions. Half-lives of exchange varying from a few hours to many days have been observed with glycero-phosphates, sugar phosphates and nucleic acids in homogeneous systems where chemical methods are used to separate the orthophosphate and organo-phosphate components. In soil systems where this physical separation may be achieved by adsorption of the organo-phosphates on the soil-mineral matrix, it is possible that the characteristics of isotopic exchange with orthophosphate ions in solution may be altered. The nett effect would be to reduce the rate of exchange which would increase the half-life under comparable conditions of

the exchange of phosphate ions between the two components. The possibility of such a mechanism occurring in soil systems must be envisaged although the present work does not provide evidence of its existence. It is clear, however, that adsorbed organo-phosphate components, if present, must occur in the 'medium' or 'slow' fractions because of the magnitude of the half-lives of exchange.

SUMMARY

The labile phosphate in a non-calcareous and a slightly calcareous soil was determined by isotopic exchange in the presence and absence of 0.001 molar solutions of a chelating and a non-chelating organic anion. The rate of isotopic exchange curves were analyzed graphically to sub-divide the labile phosphate into 3 or 4 fractions. The half-lives of exchange for the 'rapid', 'medium' and 'slow' fractions were between 0.3 to 1.6 hours, 1.8 to 8.6 hours and 25.8 to 46.1 hours respectively. In addition, an 'instantaneously-exchanging' component was sometimes observed.

In the presence of the citrate ion, the total labile phosphate was increased in the non-calcareous soil and decreased in the calcareous soil, whereas the diethyl barbiturate ion (DEB) anions decreased the total labile phosphate in both soils. In general, the citrate ion increased the 'rapid' and the 'medium' fractions whereas the DEB anion either did not affect them or decreased them. Again, whereas citrate always increased the phosphate in solution, the effect of DEB anions depended on the soil. The major effect of the organic anions was to greatly decrease the 'slowly-exchanging' fraction in both soils.

The half-lives of exchange for the 'rapid', 'medium' and 'slow' fractions were in the order

no organic anion $>$ citrate $>$ barbiturate

and the rate constants for a first-order mechanism were in the order

no organic anion $<$ barbiturate $<$ citrate.

Small but significant differences were observed between the two soils.

ACKNOWLEDGMENTS

One of us (P.A.) is indebted to the Division "Alonso de Herrera" of the Council of Higher Scientific Research, Spain, and the Ramsay Memorial Fellowships Trust for financial Support.

Received August 8, 1958. Revised draft April 8, 1959

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Plant and Soil XI, no. 4 November 1959

FACTORS INFLUENCING THE ISOTOPICALLY EXCHANGEABLE PHOSPHATE IN SOILS

PART II. THE EFFECT OF BASE SATURATION WITH SODIUM AND CALCIUM IN NON-CALCAREOUS SOILS

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The retention of phosphate ions by soils and soil-clay minerals has been related to the valency of the saturating cation (Aderikin [1] Allison [2], Barbier *et al.* [4], Pratt and Thorne [8], Wild [10]). Increased retention is invariably observed as the valency of saturating cation increases; this increase is considerably accentuated as the pH of the soil suspension is raised above 6 to 7. A cationic series has been suggested (Aderikin [1]) in the order of decreasing P-retention

$$Fe^{+++} > Ca^{++} > Mg^{++} > K^{+} > Na^{+}$$

The phosphate-ion activity in solution in equilibrium with these cation-saturated materials also depends on the activity and the valency of the cation in the solution; this may play a considerable part in the observed retention of phosphate by soils and allied materials.

The retention of phosphate by the soil and the phosphate concentration in the equilibrium soil solution are related to the 'labile phosphate' of the soil. Klechkovskii and Zherdetskaya [7] found that phosphate sorbed on potassium- and calcium-saturated forms of chernozem, peat podzol, and krasnozem soils was equally exchangeable isotopically. Little detailed work on this topic has been published. The present work examines the influence of ex-

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changeable cations on the labile phosphate by applying methods used in earlier work (Talibudeen[9], Arambarri and Talibudeen[3])

METHODS AND MATERIALS

TABLE 1

Description of soils					
Soil	pH in 0.02 *M* KCl	Total P, mg P %	Clay %	Location	Brief description
A	6.8	90.6	20	Rothamsted	Highfield
B	6.9	113.0	19	Rothamsted	Broadbalk Section V (8) unlimed
C	6.8	136.5	20	Rothamsted	Park Grass Plot 4 (i) limed
D	6.8	56.7	20	Rothamsted	Park Grass Plot 2 (i) limed
E	6.0	62.5	11	Old Kennington	Superphosphate-treated plot
F	6.8	119.0	10	Shardlow	Plot F 11. (Blood [6])
G	6.9	20.8	15	Seville, Spain	Unmanured terra rossa

Preparation of cation-saturated soils

The soils were treated repeatedly with molar solutions of NaCl and $CaCl_2$ adjusted to pH 6.8–7.0, and washed with alcohol and acetone until all chloride ions were removed. The samples were air-dried. A calcium-saturated sample of soil A using 0.01 molar calcium chloride was also prepared; analyses of the decantate showed that complete calcium saturation of 10 g soil was achieved with four 80 ml volumes of the calcium chloride solution.

Experiment 1

The total isotopic exchange was determined at isotopic equilibrium in Na- and Ca-saturated samples of soils B to G in 0.02 *M* NaCl and 0.0067 *M* $CaCl_2$ respectively; the total labile phosphate was calculated from the equilibrium phosphate concentration in solution and the fraction of P^{32} left in solution at equilibrium. Although no difficulty was experienced in separating the soil from the solution by centrifugation at high speeds, the solutions from the Na-soil experiments (0.02 *M* NaCl) mostly had a faint, brownish yellow tinge which could be attributed to the partial peptisation of the fine fraction in the soil. This would not affect the P^{32}-content of the solutions determined by counting but may give a positive error in their total phosphorus contents by the molybdenum-blue method.

For comparison, labile-phosphate values were also similarly determined in untreated samples of soils A to G suspended in 0.02 *M* KCl.

Experiment 2

Using methods described earlier (Arambarri and Talibudeen[3]) the isotopic exchange of orthophosphate ions in Na- and Ca-saturated samples of

soil A in equilibrium with 0.02 *M* NaCl and 0.0067 *M* $CaCl_2$ respectively, was determined at 25° and 35° C.

RESULTS AND DISCUSSION

EXPERIMENT 1

Klechkovskii and Zherdetshaya [7] showed that phosphate ions fixed on calcium and potassium saturated soils are equally capable of isotopic exchange. Table 2 gives the distribution of the

TABLE 2

The labile phosphate in mg P per 100 g soil in sodium- and calcium-saturated soils * and its partition between the soil and the solution at 22–25° C and a dilution of 1 : 200 **

Soil	Na-soil in 0.02 *M* NaCl		Ca-soil in 0.0067 *M* $CaCl_2$		Untreated soil in 0.02 *M* KCl	
	P in solution	Total labile P_e	P in solution	Total labile P_e	P in solution	Total labile P_e
A	3.1	13.3	0.62	11.1	0.8	11.3
B	17.3	33.5	3.0	24.7	8.7	30.5
C	22.6	35.7	6.6	31.7	20.0	44.2
D	0.6	5.3	0.1	6.6	0.3	4.3
E	8.1	12.8	2.1	11.8	6.7	13.7
F	17.0	30.7	2.5	20.6	9.2	27.2
G	0.9	2.0	0.1	1.6	0.6	1.7

* Saturated by washing with molar solutions of sodium and calcium chlorides as described in "Methods".

** These exchanges were carried out in solutions of equal ionic strength $\mu = 0.02$ at a dilution of 0.5 g soil in 100 ml solution.

total labile phosphate P_e into the phosphate in solution P_{ss} and on the soil surface P_s for soils A to G. Results are given separately for the sodium- and calcium-saturated soils and for the untreated soils. The labile phosphate in six of the seven sodium-saturated soils is higher than in their calcium-saturated forms. This may be attributed partly to the contamination of the isotopically exchangeable phosphate in the solution in equilibrium with sodium-saturated soils by non-exchangeable (partly organic) forms of phosphate. If the constancy of the product of the concentrations of cations and H_2PO_4-ions can be taken as a guide to the relative magnitudes of

the concentrations of orthophosphate ions in the NaCl and $CaCl_2$ solutions

$$i.e.\ (M)^{\frac{1}{v}}.(H_2PO_4) = \text{constant}$$

where v is the valency of the cation M,

the 'true' orthophosphate concentration in the NaCl solutions can be calculated using that in the $CaCl_2$ system as a reference. Using the observed partition coefficient of radioactivity in the Na-system, its 'true' labile phosphate can now be calculated and compared with the values for the Ca-systems. The results of these computations are given in Table 3, which shows that for soils B and F

TABLE 3

Comparison of calculated and observed $[H_2PO_4]$ concentrations and labile-phosphate values in sodium soils with values in calcium-saturated soils

Soil	$[H_2PO_4]$ concentrations * in molarity × 10^7			Labile-phosphate values (P_l) in mg per 100 g soil		
	NaCl Solution		$CaCl_2$ solution	Na-system		Ca-system
	Observed	Calculated		Observed	Calculated	
A	31.3	25.4	6.2	13.3	10.8	11.1
B	173.0	123.0	30.1	33.5	23.8	24.7
C	227.0	271.0	66.2	35.7	42.7	31.7
D	6.4	4.7	1.1	5.3	3.9	6.6
E	80.9	87.5	21.4	12.8	13.8	11.8
F	171.0	103.0	25.2	30.7	18.6	20.6
G	8.2	4.1	1.1	2.0	1.0	1.6

* Using a pk'' value of 7.016 at 25° C for phosphoric acid at $\mu = 0.02$ (Bjerrum[5]).

where a marked difference is observed between the values for sodium and calcium systems, the calculated values for the sodium systems are similar to the observed values for the calcium system. In soil C, however, the $[H_2PO_4]$ concentration observed in solution is much lower than the calculated value, suggesting the presence of a multivalent cation *e.g.* $[Al(OH)]^{++}$ or $[Fe(OH)]^{++}$ in the NaCl extract. Expressing the distribution of phosphate between the observed phosphate in solution and in the soil by a partition ratio:

$$\frac{\text{P in solution}}{\text{Labile P in soil}},$$

gives a ratio in the sodium systems 5 to 9 times higher than in calcium systems. Thus it appears that the total labile phosphate computed from isotopic-exchange experiments is not sensitive to

the valency of the saturating cation provided appropriate correction can be made for non-isotopically exchangeable forms of phosphate in the solution.

Influence of pre-treatment for base-exchange saturation

These soils were also examined without pretreatment with saturating solutions. A comparison of the isotopic exchange data in the treated and untreated soils is of considerable interest (Table 2). The untreated soils were suspended in 0.02 M KCl to determine the labile phosphate, the number of K-ions in solution thus being considerably greater than the exchangeable cations in the soil. Even if the exchangeable cations were predominantly divalent, a fair degree of cation exchange is to be expected with the formation of a mixed ($Na^+ + K^+ + M^{++}$) soil containing an increased percentage of exchangeable, monovalent potassium ions, the properties of this system being intermediate between those of the Na- and Ca-systems. A comparison of the data show that this is realised in the magnitudes of the phosphate in solution; in fact, the phosphate concentration of the solution in equilibrium with the untreated soil indicates the valency of the predominating exchangeable cation in the soil. For instance a comparison of soils D and G shows that their P-concentrations in the Na- and Ca-systems are very similar. The P-concentration for the untreated soil is more than double that of soil D suggesting that the predominating, exchangeable cations in the two untreated soils are monovalent and divalent respectively.

Generally the labile phosphate in the untreated soils and the Na- and Ca-saturated soils are of the same order even when no corrections are made for the non-isotopically exchangeable phosphate in solution.

In the following section the influence of the valency of the cation, of temperature, and of the concentration of the saturating solution of calcium chloride on the distribution of the components of the labile phosphate and their rates of isotopic exchange in soil A are examined using methods described earlier (Arambarri and Talibudeen [3]). The labile phosphate P_s in the soil can be divided into three or four fractions P_0, P_1, P_2 and P_3 according to the speed with which they can exchange phosphate ions isotopically with the phosphate in solution P_{ss}.

EXPERIMENT 2

Valency of saturating cation

The labile phosphate P_s left in the soil after the extraction of phosphate by the equilibrium solution increases in going from sodium to calcium saturation due to the decrease in the phosphate in solution P_{ss} (Table 4). The rates of exchange for the 'rapid'

TABLE 4

The distribution of the labile phosphate P_e into its components in sodium and calcium forms of soil A and the effect of temperature on this distribution
Units: *Amounts* mg P per 100 g soil;
Rate of isotopic exchange in mg P per 100 g soil per hour

Components	Sodium-saturated Soil				Calcium-saturated Soil			
	25° C		35° C		25° C		35° C	
	Amount	Rate	Amount	Rate	Amount	Rate	Amount	Rate
'Rapid', P_1	2.2	Fast	2.1	Fast	2.9	Fast	4.3	Fast
'Medium', P_2	1.4	0.22	1.5	0.38	0.3	0.05	0.3	0.05
'Slow', P_3	6.6	0.04	10.4	0.079	7.4	0.01	12.6	0.008
Total in Soil, P_s	10.2	—	14.0	—	10.5	—	17.2	—
'Solution', P_{ss}	3.1	—	3.4	—	0.6	—	0.6	—
Total in system, P_e	13.3	—	17.3	—	11.1	—	17.8	—

fraction are not changed significantly at 25° or 35° C by Na- and Ca-saturation, but the rates for the medium and slow fractions are between 4 and 10 times greater at 25° and 35° C respectively, in the Na-system. This suggests that there is an intimate linkage between the exchangeable calcium ions and phosphate ions in the 'medium' and 'slow' components, and that the 'rapid' component is relatively insensitive to the exchangeable cation. The 'medium' and 'slow' components together constitute from 73 to 85 per cent of the labile phosphate left in the soil, thus this behaviour seems to support the hypothesis of a calcium–phosphate complex being formed in the surface-adsorbed phosphate in which the exchangeable calcium ions play an important role.

Influence of temperature

Table 4 shows that the total labile phosphate in the Na- and Ca-systems increases appreciably with a 10° C rise in temperature, but whereas the phosphate in solution increases by about 1 per cent per °C in the Na-system, there is no significant change in the phosphate

concentration of the solution in the Ca-system with temperature. The increase in the labile phosphate at 35° C shows clearly in the 'slow' phosphate, although in the Ca-soil there is also a small increase in the 'rapid' fraction. The difference in the total labile phosphate between the two systems at 25° C is removed by increasing the temperature to 35° C.

It was shown earlier that substituting sodium ions for calcium ions in base-exchange positions did not affect the rate of the 'rapid' exchange but increased the rates of the 'medium' and 'slow' exchanges. The influence of temperature also suggests a change in the nature of the linkage between the exchangeable cations and adsorbed phosphate in going from Ca^{++} to Na^{+} ions. Here again, there is no significant change in the rate of the 'rapid' exchange in the Na- or Ca-systems, but whereas in the Na-systems the rates of 'medium' and 'slow' exchanges are doubled with a 10° C rise in temperature the corresponding values in the Ca-system are unaltered. This behaviour strongly supports the conclusion arrived at in the previous section that the exchangeable calcium ions are more strongly linked than sodium ions with the 'medium' and 'slow' exchanging phosphate ions and that the linkage is not temperature-sensitive in the range 25° to 35° C.

Influence of the concentration of $CaCl_2$ solution used for base saturation of the soil

As exchangeable calcium ions affected the magnitude and the behaviour of the 'medium' and 'slow' components of the labile phosphate, it was thought necessary to investigate whether this was due to the precipitation of a calcium-phosphate compound on the surface of the soil during the procedure used for base saturation with molar calcium chloride. From the known solubility products of calcium phosphates (Bjerrum [5]), a simple calculation shows that a 0.01 *M* $CaCl_2$ could not precipitate any of these compounds in these soils. Table 5 shows the isotopic exchange for soil A, saturated with calcium ions by 0.01 *M* and 1.0 *M* $CaCl_2$ solutions. These results clearly demonstrate that the 'medium' and 'slow' components are unaffected by the concentration of the saturating solution either in magnitude or in the rate at which they exchange. This emphasises the fact that the differences observed between the rates of exchange and between the phosphate exchanging

TABLE 5

The labile phosphate and its components at 25° C in the calcium-saturated form of soil A in equilibrium with 0.0067 *M* $CaCl_2$
Units: *Amounts* mg P per 100 g soil;
Rate of isotopic exchange in mg P per 100 g soil per hour

Components	Base saturation with 0.01 *M* $CaCl_2$		Base saturation with 1.0 *M* $CaCl_2$	
	Amounts	Rate	Amounts	Rate
'Rapid' P_1	4.3	Fast	2.9	Fast
'Medium' P_2	0.3	0.04	0.3	0.05
'Slow' P_3	8.3	0.009	7.4	0.01
Total in Soil P_s	12.9	—	10.5	—
Solution P_{ss}	0.4	—	0.6	—
Total in System P_e	13.3	—	11.1	—

isotopically in sodium- and calcium-saturated soils can not be caused by precipitated calcium phosphates in the latter.

Wild [10] showed that in an aluminium-saturated resin, foreign cations added to the system influence the reaction between added phosphate and the aluminium according to their valency and the size of their hydrated forms. He suggests that the retention of phosphate by clays due to this reaction differs only in degree from that of the resin; he advances two alternative explanations for this behaviour namely that the exchangeable cations influence (*i*) the distribution of phosphate ions within the diffuse part of the electrical double layer associated with the resin or clay and (*ii*) the amount of reactive aluminium released from the resin or clay. Although the evidence given by Wild for the role of reactive aluminium is not entirely conclusive, it plausibly explains the decreased mobility of the more slowly exchanging forms of phosphate in calcium-saturated clays. Base saturation of the clay with a cation of higher valency would increase the activities of free aluminium and phosphate ions and bind the adsorbed phosphate more strongly due to increased interaction between the two ions. The degree of reversibility of such an interaction may be partly responsible for the anomalous behaviour of the soil phosphate *e.g.* the changes observed in the equilibrium phosphate concentrations in the soil solution after the consecutive "wetting" and "drying" of the soil.

SUMMARY

The effect of base saturation of non-calcareous soils with sodium and calcium on their total labile phosphate was studied in solutions of sodium

and calcium chlorides of the same ionic strength ($\mu = 0.02$). The total labile phosphate in 6 out of 7 soils was between 8 to 40 per cent higher in sodium-saturated soils than in calcium-saturated soils. A much larger part of the total labile phosphate was found in solution in the sodium-saturated soils. The ratio of phosphate in solution in these soils to that in calcium-saturated soils varied from 3.4 to 8.1 with a mean value of about 5.5 as compared with a theoretical value of 4.1. A method is suggested for obtaining 'corrected' values for the abnormally high phosphate concentrations in solution from sodium-saturated soils. 'Corrected' values give smaller differences between the total labile phosphate in sodium and calcium forms of these soils.

Rates of isotopic exchange of the more slowly exchanging forms of soil phosphate were measured at 25° and 35° C for the sodium- and calcium-saturated forms of one Rothamsted soil. At 25° C these rates were four times higher in the sodium-saturated soil than in the calcium-saturated soil although the total labile phosphate in the two forms was the same. Increasing the temperature to 35° C doubled these rates of exchange in the sodium-saturated soil but had no effect on the calcium-saturated soil. This suggests that the cation-exchangeable calcium is strongly linked to the slowly-exchanging forms of phosphate in the soil.

No differences were observed in the rates of isotopic exchange of phosphate in this soil after calcium saturation with either 0.01 molar or 1.0 molar calcium chloride. The precipitation of calcium phosphates during the calcium-saturation procedure could not therefore be responsible for the observed differences between sodium- and calcium-saturated samples.

Received April 8, 1959

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Plant and Soil XI, no. 4 November 1959

FACTORS INFLUENCING THE ISOTOPICALLY EXCHANGEABLE PHOSPHATE IN SOILS

PART III. THE EFFECT OF TEMPERATURE IN SOME CALCAREOUS SOILS

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INTRODUCTION

When results for the rate of isotopic exchange in two Rothamsted soils were analysed graphically, the *total* isotopically exchangeable (or 'labile') phosphate of a soil suspended in 0.02 *M* KCl solution was found to be subdivisible into three or four fractions depending on the rates at which these fractions exchanged isotopically with the phosphate ions in solution [10]; the slowly exchanging part of the isotopically active phosphate in the calcareous soil was decreased by introducing low concentrations of organic anions although its lability was increased.

We now report the rate of dissolution of the ortho-phosphate ion, the equilibrium concentration of phosphate ions in solution, the rate of isotopic exchange, and the total isotopically exchangeable phosphate at 25°, 35° and 45°C, in four soils of similar mechanical composition but with varying amounts of calcium carbonate, total phosphorus and organic carbon.

Aslyng [1] observed that with Rothamsted soils (pH range 4.5–7.5) the equilibrium phosphate concentration in 0.01 molar calcium chloride increased 1 to 2 per cent per degree centigrade as the temperature was raised from 9° to 27°C. He also showed that this effect was reversible between 0° and 30°C, suggesting that the soil phosphate does not change irreversibly within this

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temperature range. Our work is confined to the examination of calcareous soils where the presence of the ortho-phosphates of calcium will mainly contribute to the behaviour of the soil phosphate. Although much detailed work has been done on the system $CaO–P_2O_5–H_2O$, the effect of temperature on its kinetics has not been clearly defined. The results obtained by Warington [11] and later Bassett [3] suggested that the hydrolysis of solid calcium ortho-phosphates in aqueous suspension leads to the loss of phosphorus or calcium to the solution until a solid with a Ca : P ratio of 1.67 is obtained. Schleede *et al.* [9] demonstrated this clearly but they also found that dicalcium phosphate dihydrate was hydrolysed to an apatite-like material only after a prolonged hydrolysis with *"hot"* water. The rate of this hydrolysis increased when a critical amount of a solid calcium phosphate more basic than the dicalcium phosphate had been formed [5 8]. Their observations provide a logical explanation for the difference between the behaviour of phosphate-rich and phosphate-deficient calcareous soils observed in the work described below.

The kinetics of the dissolition of phosphate at a fixed volume and a constant ratio of soil: solution have not been studied, presumably because the solid phase is so complex. If, however, it is assumed that the rate-controlling process is the diffusion of ions from the surface through a constant thickness of the "saturation layer" of solution, the rate of dissolution of phosphate is proportional to the distribution of phosphate on the soil surface and to the saturation deficiency in solution. (The saturation deficiency is defined as the difference between the concentrations at saturation and at any time before saturation). A comparison of the rates of dissolution at different temperatures at the same saturation deficiency would show whether any changes occurred in the surface distribution of phosphate. We have used this concept to interpret qualitatively the differences observed between the kinetics of phosphate dissolution from phosphate-rich and phosphate-deficient calcareous soils.

Little is known about how temperature affects the isotopic exchange of phosphate ions in soils, but it is generally assumed that increasing temperature must increase the rate of isotopic exchange. Bandrenghien and Govaerts [4] measured the rates of isotopic exchange of both calcium and phosphate ions in solid 'tricalcium

phosphate' suspended in $CaCl_2$ and Na_2HPO_4 solutions at temperatures between 18° and 180°C. They found that the rate of exchange for both ions increased to the same extent up to 60°, after which the rate of phosphate exchange increased continuously up to 180° whereas the rate of calcium exchange increased at a rate greater than that of phosphate. They found that at temperatures greater than 60° the solid phase was converted to a chlorapatite and suggested that the calcium exchanged faster because calcium ions and their supplementary chloride ions migrated into the crystalline solid (in equivalent exchange for ionic $Ca(OH)_2$). Our work shows that in calcareous soils of low phosphate status similar discontinuously large changes occur in the rate of phosphate exchange at temperatures greater than 35°C; presumably, they are associated with a change in the nature of the solid phosphate in the soil.

MATERIALS AND METHODS

The arable soils used are described in Table 1; soils C and D have a high phosphate status relative to soils A and B. The mechanical compositions of the soils are approximately the same, the major differences being in their

TABLE 1

Description of soils								
Soil number	Rothamsted number	Location and description	pH in 0.02*M* KCl	Clay content	$CaCO_3$ %	Organic carbon * %	Total ** phosphorus mg P per cent	Response to phosphate fertilisation
A	A6480	Soke of Peterborough; Light loam on Lincolnshire limestone.	7.7	22.6	3.2	2.10	52.6	Good
B	A8832	Rothamsted; Exhaustion land ("Superphosphate" plot)	7.6	19.3	1.5	1.01	66.2	Moderate
C	A8867	Rothamsted; Highfield .	6.8	20.3	<0.1	3.63	96.4	None
D	A6491	Midland Agricultural College Centre, Tinwell, Rutland; Medium loam on Inferior Oolite	7.9	18.5	23.1	2.65	156.0	None

* By dry combustion method

** By $HClO_4$ digestion

total phosphate and $CaCO_3$ contents. Soils C and D contain much more phosphate than soils A and B.

The methods used were similar to those already described [10]; 0.5 g of the soil was shaken continuously with 100 ml of 0.02 *M* KCl at constant temperature and the phosphate dissolved from the soil was determined at suitable intervals. The suspension was labelled with "carrier-free" * P^{32} when the initial rapid rise in phosphate concentration had ended. The total phosphate and P^{32} concentration in solution were measured at suitable times until the specific activity in solution was constant within experimental error. Care had to be taken with centrifugation at high speeds when separating the soil and the solution to keep the system at chemical equilibrium at the time of sampling. The whole sampling procedure required about 15 minutes.

RESULTS

Preliminary experiments on the rate of dissolution of phosphate and its temperature dependence

The general pattern for the rate of dissolution of phosphate ions

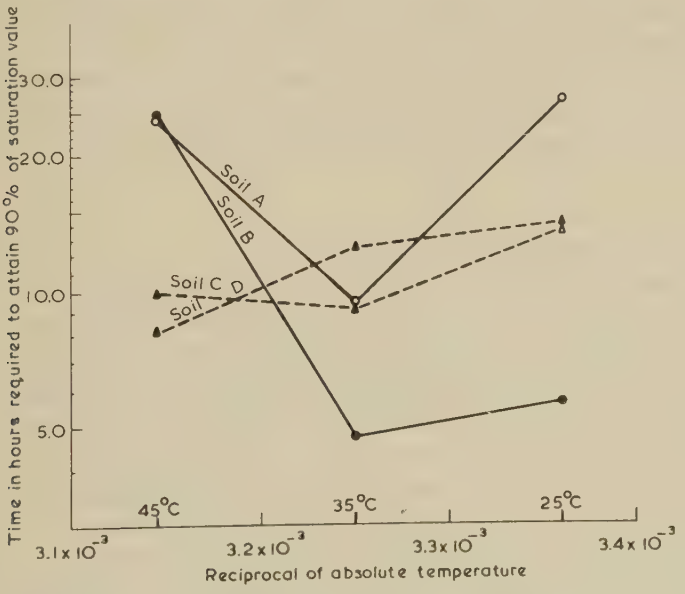

Fig. 1. Influence of temperature on the rate of dissolution of phosphate from 0.5 g soil in 100 ml 0.02 *M* KCl.
Low P status ———, High P status – – – –.

* The term "carrier-free" implies that the phosphate concentration of the radioactive solution added was too small to change the phosphate concentration of the soil solution measurably.

for these soils exhibited two characteristics – an initial rapid increase with time in the phosphate removed from the soil, followed by a slow increase or a slow decrease at longer times. Soils A and B, with low total phosphate, showed a slight increase, which was little affected by temperature. It is assumed here that the rapid increase with time corresponds to a true dissolution and the subsequent behaviour comes from secondary unspecified reactions. The time when the rapid increase ends was estimated graphically, and the amount of phosphate dissolved at this time was taken as the saturation value for the "true dissolution" process. This saturation point was attained more rapidly as the temperature was raised from 25° to 35°C but the behaviour of the soils at 45°C varied according to their phosphate content. In Figure 1, the logarithm of the time required for the 90% saturation value (or 10% saturation deficiency) to be reached is plotted against the reciprocal of the absolute temperature. Soils A and B show a decrease in the rate of dissolution (corresponding to an increase in the time required for 90% exchange) above 35°C unlike soils C and D which contain much more phosphate; thus soil C does not show any change in the rate of dissolution between 35° and 45°C, and soil D, with a considerably higher P reserve, shows a normal increase in the rate of dissolution with temperature.

Influence of temperature on the equilibrium concentration of phosphate in the soil solution

The only systematic observations on the influence of temperature on the equilibrium concentration of phosphate in the soil solution in the literature appear to be those of Aslyng [1] already referred to. These show that in Rothamsted soils there is a regular and reversible increase of 1 to 2 per cent per degree centigrade in the phosphate concentration with increasing temperature in the range 0° to 30°C. In Figure 2, the logarithm of the equilibrium of phosphate concentration is plotted against the reciprocal of the absolute temperature, which shows an almost linear increase from 25° to 45° in soils C and D of high P status. However, the percentage increase with soil C is much greater than that found by Aslyng, presumably because it contains much organic matter. With soils A and B of low P status, the observed increase between 25° and 35° is within the limits observed by Aslyng; between 35° and

45°, the phosphate concentration increases abnormally, suggesting a change in the nature of the solid phosphate phase.

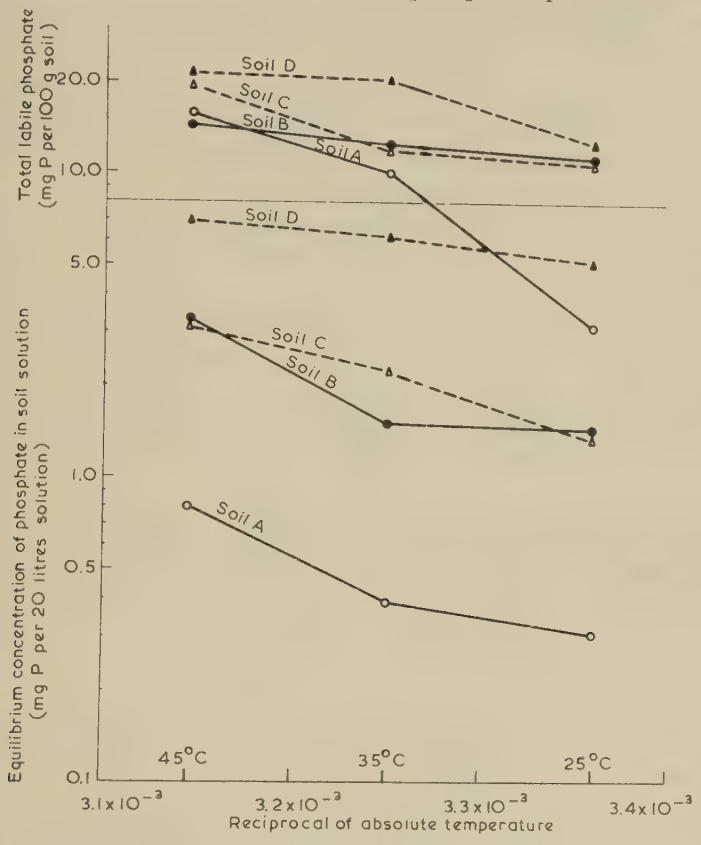

Fig. 2. Influence of temperature on the total labile phosphate and the equilibrium concentration of phosphate in the soil solution.
Low P status ———, High P status – – – –.

Influence of temperature on the total labile phosphate

The total labile phosphate in all the soils increases with temperature (Figure 2) and, except for soil A, this increase is relatively small and regular between 25° and 45°. The total labile phosphate in soil A, which has the lowest phosphate content of the four soils, increases considerably but fairly regularly in this temperature range. Apart from this, soils of low and high phosphate status show no clear difference in the behaviour of their total labile phosphate.

The labile phosphate can be sub-divided into the 'instantaneous', 'rapid', 'medium' and 'slow' components as described previously [10]. The influence of temperature on the distribution of these components shows the following trends:

(1) As the temperature of exchange is increased, it becomes increasingly difficult to separate the 'instantaneous' and 'rapid' exchanging components; these are, therefore, referred to here as a single 'rapid' exchanging component.

(2) The increase in the total labile phosphate and the phosphate in solution is reflected in a corresponding increase in the 'rapid' component.

(3) The magnitude of the 'medium' component is always very small and does not show any regular alteration with temperature.

4() The 'slow' component, however, is always large and tends to decrease with increasing temperature *except* in soil A, which shows a large increase as the temperature is raised from 35° to 45°C.

Influence of temperature on the rate of isotopic exchange

As shown previously [10], the rate of isotopic exchange is best represented by a rate constant from the relation:

$$R_x = \frac{0.693}{t_{\frac{1}{2}}} \cdot \frac{P_x . P_{ss}}{P_x + P_{ss}}$$

where P_x is the isotopically-exchangeable phosphorus in the 'rapid', 'medium' and 'slow' exchanging components *respectively* of the labile phosphate P_s in the soil, P_{ss} is the phosphate in solution and $t_{\frac{1}{2}}$ is the half-time of exchange graphically determined from the linear variation of the logarithm of the fractional exchange with time.

Thus $P_s = P_1 + P_2 + P_3$ and the total labile phosphate $P_e = P_s + P_{ss}$.

Table 2 shows the influence of temperature on the magnitudes and the rate of exchange of the 'rapid', 'medium' and 'slow' components for four soils. These results clearly illustrate (1) the increase in the rates of exchange with temperature, (2) the difference in the behaviour of soils of low and high phosphate status between 35° and 45°C.

The rate of the 'rapid' exchange is usually too fast to measure accurately. The magnitude of the 'rapid' component increases

TABLE 2

Influence of temperature on the components of the total labile phosphate in four soils (Magnitude of components in mg P per 100 g soil: Rates R_x in mg P per 100 g soil per hour)

Component of total labile phosphate	Temperature	Soil A		Soil B		Soil C		Soil D	
		Component	Rate	Component	Rate	Component	Rate	Component	Rate
'Rapid' P_1	25°	0.81	*	1.69	*	1.94	*	0.86	0.84
	35°	3.17	*	2.22	*	4.62	*	4.54	2.53
	45°	4.34	*	8.49	*	8.44	*	6.42	2.23
'Medium' P_2	25°	0.10	0.011	0.43	0.070	0.42	0.022	1.04	0.18
	35°	0.12	0.053	0.48	0.047	0.21	0.048	2.82	0.45
	45°	0.33	0.089	0.39	0.215	1.21	0.177	1.91	0.34
'Slow' P_3	25°	2.10	0.0035	7.74	0.021	8.16	0.013	8.72	0.046
	35°	6.31	0.015	8.04	0.027	6.15	0.079	8.34	0.078
	45°	10.50	0.116	2.99	0.139	7.25	0.069	7.25	0.092
Total in soil P_s	25°	3.01	—	9.86	—	10.52	—	10.62	—
	35°	9.60	—	10.74	—	10.98	—	15.70	—
	45°	15.17	—	11.87	—	16.90	—	15.58	—
P in solution P_{ss} at time of tagging	25°	0.15	—	1.65	—	0.80	—	2.04	—
	35°	0.44	—	1.74	—	1.34	—	4.96	—
	45°	0.55	—	2.69	—	2.58	—	5.08	—
Total labile P_e phosphate	25°	3.16	—	11.51	—	11.32	—	12.66	—
	35°	10.04	—	12.48	—	12.32	—	20.66	—
	45°	15.72	—	14.56	—	19.48	—	21.66	—

* The rate of exchange was too fast to be measured reliably.

gradually in all the soils between 25° and 45° and no clear distinction can be drawn between soils of low and high phosphate status. This is parallel to the behaviour of the total labile phosphate described earlier.

The magnitude of the 'medium' component is invariably very small and in general shows little significant change with temperature. The rate of exchange, however, shows an abnormal increase with soils A and B between 35° and 45°C.

The 'slow' component P_3 always forms a significant part of the labile phosphate. Its magnitude does not vary appreciably with temperature with soils C and D of high phosphate status but in soil A, P_3 increases considerably between 25° and 45° in contrast

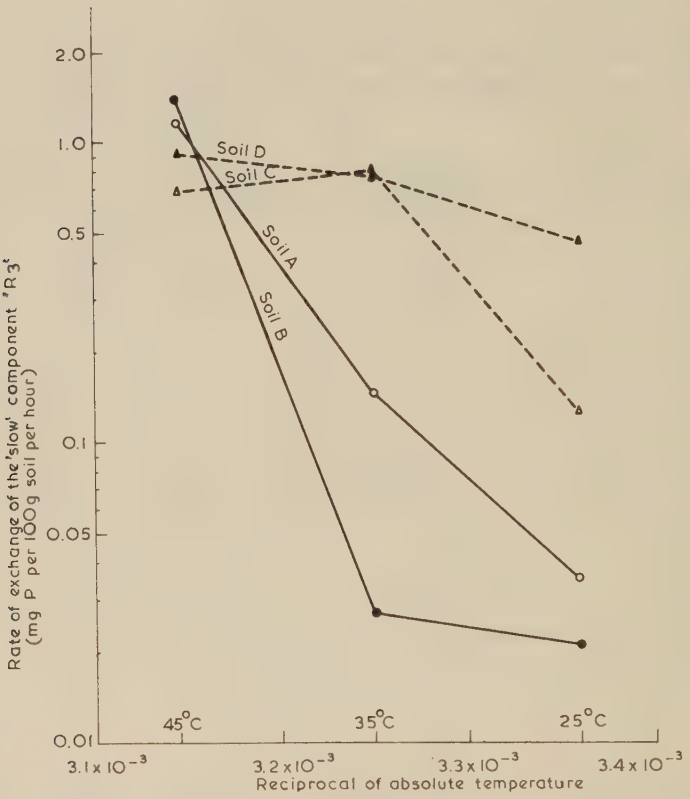

Fig. 3. Influence of temperature on the rate of exchange of the 'slow' component 'R_3'.
Low P status ———, High P status – – – –.

with the behaviour of P_3 in soil B. The rate constant for the 'slow' exchange (Table 2 and Figure 3) for all soils between 25° and 35°C, and for soils C and D from 35° to 45°, increases slightly. Soils A and B, however, show a very sharp increase in the rate constant between 35° and 45°, although the magnitudes of their 'slow' components differ vastly. The rate constants at 45° in soils A and B of low P status are of the same order (0.116 and 0.139 mg P per 100 g soil per hour) and are significantly greater than those in soils C and D (0.069 and 0.092 mg P per 100 g soil per hour) of high P status.

DISCUSSION

The principal feature of these results is the difference in the behaviour of soils of low and high phosphate status between 35° and 45°C. Thus, with soils A and B, the effect of temperature on the rates of dissolution, on the equilibrium concentration of phosphate, and on the rate of exchange for the 'slow' components, suggests the conversion of their 'slow' component into a new phosphate phase.

The inorganic phosphate in calcareous soils in aqueous suspension thus seem to be in two distinct parts – a small but nevertheless important amount, which is rapidly labile, is probably associated with the surfaces of the clay and to a lesser extent with the calcium carbonate surfaces; the remainder, which exchanges slowly, consists of a mixture of the insoluble orthophosphates of calcium whose composition, and hence whose subsequent behaviour, depends on the conditions under which they were precipitated. Although the nature of these calcium phosphates must be independent of the amount of $CaCO_3$, they must be intimately associated physically with the calcium carbonate in the soil which is the source of the calcium concentration in the soil solution. The *amount* of $CaCO_3$ in the soil must thus control the *amount* of the insoluble calcium orthophosphate formed. The nature of these phosphates depends on the activity of calcium and phosphate ions in solution, the former being almost independent of the amount of $CaCO_3$ present. In phosphate-rich calcareous soils, where the activity ratio $A_{Ca} : A_P$ is low, the principal component of the precipitated phase will be dicalcium phosphate contaminated with a calcium-deficient apatite (*i.e.* an apatitic structure with an atomic ratio Ca : P less than 1.67) whose amount and nature will depend on the amount of the soluble phosphate added initially to the soil. These results suggest that in such soils (C and D) this apatite is less than the critical amount suggested by the work of Buch [5] and Sanforche and Henry [8]; accordingly the rate of hydrolysis of the dicalcium phosphate to an apatite phase is very small and is indeed not measurable in our experiments. The reverse holds for the phosphate-deficient soils A and B where the clearly measurable changes in the slowly labile phosphate with temperature point to the rapid hydrolysis of the precipitated calcium phosphates and

the presence of a much higher proportion of a *calcium-deficient* apatite, which is rapidly hydrolysed at higher temperatures to a true apatite with the consequent production of a phosphate-rich solution. It follows that, when the phosphate activity in the soil solution is continuously depleted by some external agency (*e.g.* by plant roots or by continuous rapid leaching), the nature of the dicalcium phosphate residue in phosphate-rich calcareous soils will not be significantly affected until it is decreased to a very small proportion. By contrast, in a phosphate-deficient soil, such a process will considerably hasten the hydrolysis of the calcium-deficient apatite to a much less labile true apatite.

Aslyng [2] suggested that some calcareous Rothamsted soils contain octocalcium phosphate [5]. The hydrolysis of octocalcium phosphate at high temperatures leads to the formation of hydroxyapatite *and* dicalcium phosphate, probably according to

$$2Ca_4H(PO_4)_3 + H_2O \rightarrow Ca_5(PO_4)_3.OH + 3CaHPO_4$$

Octocalcium phosphate is itself produced by the hydrolysis of dicalcium phosphate and a cyclic process can be imagined in which the octocalcium phosphate residues in the soil are progressively converted to hydroxyapatite. Until this conversion is nearly complete, the more soluble dicalcium phosphate may govern the 'quasi-equilibrium' concentrations in the soil solution and cause the abnormal behaviour of calcareous soils of low P status.

The presence of potassium chloride offers another explanation of this behaviour in soils A and B. The chloride ion diffuses fairly readily at elevated temperatures as a counter-ion (Bandrenghien and Govaerts [4]) or in isomorphous substitution (for OH-ions) into apatite or pseudoapatite type of phosphates to form a chlor-apatite-like phosphate (Bjerrum [5]) with greatly increased solubility and lability. It is significant that soils C and D (the latter having 23 per cent calcium carbonate) show no signs of this behaviour, which suggests that if they contain any apatite-like or allied phosphates these cannot be detected in the 'labile' phosphate, and that their behaviour is masked by that of phosphate compounds of higher energy content.

The increased uptake of phosphorus by plant roots at elevated temperatures has been ascribed (Zhurbitzky and Shtrausberg [7]) to increased plant-physiological activity in the roots as well as

in the tops of plants. It is clear from this work that the higher rates of dissolution and the higher equilibrium concentrations in the soil as well as an increased rate of turnover of the labile phosphate in the soil may, at least, be equally responsible for increased phosphate uptake.

The turnover of the 'slow' component in soils of high P status is 5 to 10 times faster than in soils of low P status at 35°C; but at 45°C, the situation is reversed, the rates in the low-phosphate soils being $1\frac{1}{2}$ to 2 times higher than in soils of high P status. This suggests that an abnormally high uptake of phosphate may be realised from calcareous soils of low phosphate status et temperatures above 35°C.

SUMMARY

The influence of temperature in the range 25° to 45°C on the rate of dissolution and the equilibrium concentration of phosphate, on the labile phosphate and its components, and on the rates of isotopic exchange in four soils with varying $CaCO_3$ contents and phosphate status was investigated. There were abnormal increases in the equilibrium phosphate concentration and the rate of exchange of the slowly labile phosphate of soils of low P status between 35° and 45°. The slowly exchanging component normally constitutes the greater part of the labile phosphate of the soil. The soils of higher phosphate status, irrespective of their $CaCO_3$ content, did not show this abnormal behaviour. The observed difference is connected with the nature and the composition of the basic calcium phosphates associated with the soil calcium carbonate.

Received July 23, 1959

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Plant and Soil IX, no. 4 November 1959

POPULATIONS MICROBIENNES DES BOIS

III. HUMIFICATION *IN VITRO* D'UNE SCIURE DE HÊTRE

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1. INTRODUCTION

En 1955, Lenglen, dans une note présentée à l'Académie d'Agriculture envisageait l'emploi de sciures comme sources d'humus, mais sa suggestion était accueillie avec scepticisme. Pourtant, vers la même époque, aux États-Unis, divers auteurs (Davey [17], Lunt [25], Baker et Dunn [3], *etc.*) montraient que les déchets de bois présentaient un certain intérêt à condition de les additionner d'une source d'azote et, au besoin, d'autres substances (P, K). Cependant, leur action, par ailleurs sans danger, restait lente et généralement faible. Davey [8] signalait aussi l'influence de *Coprinus ephemerus* (Il s'agissait en réalité d'une association comprenant cette espèce) susceptible de donner en quelques mois à la sciure l'aspect d'un "mull riche".

Pour notre part, nous étudions depuis plusieurs années la transformation de tas de copeaux entreposés sur les alluvions calcaires de la Meuse et chez lesquels on peut noter une humification lente, certes, mais complète. Nous en décrirons ailleurs l'histoire; disons seulement ici que la substance est transformée en un terreau brun au bout de 7 – 8 ans et en humus noir après 10 ans, avec des taux d'acides humiques variant entre 2 et 3 pour cent.

C'est pour expliquer la production de ces substances que nous avons entrepris les études relatées dans ces pages. Divers auteurs ont vu dans les lignines la principale source de substances humiques; il y a, en effet, entre ces deux groupes de composés des analogies structurales frappantes (Breger [5]) .De plus, bien souvent, le taux

de lignine s'abaisse en même temps que s'élève la teneur en acides humiques et Scheffer et Welte [32] ont proposé deux schémas de formation de l'humus, l'un en milieu acide, comportant la dégradation enzymatique de la lignine et la formation d'acides humiques "bruns", l'autre, en milieu alcalin ou neutre, consistant en une transformation, peut-être abiotique, de la lignine libérée par les microorganismes. Flaig *et al.* [11] font état de transformations progressives de la fraction "lignine" des résidus végétaux dont la teneur en méthoxyle diminue tandis que la richesse en N augmente au cours de l'humification. Notons toutefois que les méthodes de dosage de la lignine sont en partie conventionnelles et que certains protéides difficilement hydrolysables pourraient fausser les résultats.

Cependant on ne saurait mettre en doute le rôle de la lignine, même si elle ne nous apparaît pas comme la seule source d'humus. Par contre on n'est pas parfaitement renseigné sur le mécanisme de son humification: transformation directe ou dégradation profonde (Bremner [6]) avec libération de polyphénols suivie de recondensation (cf. Kononova [20]). Dans cette deuxième hypothèse, les fragments de la molécule de lignine interviendraient au même titre que les pigments quinoniques microbiens. Mais il a été impossible jusqu'ici de démontrer directement la réalité de l'un ou l'autre de ces processus, notamment parce que la lignine isolée n'est plus guère sujette à la décomposition dans les conditions biologiques (Wehrmann [35]).

Assez curieusement, certains pédologues, surtout ceux qui accordent à la lignine une importance primordiale, tendent à minimiser le rôle des germes qui la décomposent, peut-être parce que les analyses microbiologiques des sols mettent rarement ces espèces en évidence. Le microbiologiste sait que cette rareté apparente est le fruit de l'imperfection de ses méthodes et que dans les milieux assez riches en matière organique, il y a normalement une phase, précoce ou tardive, où les Basidiomycètes lignivores jouent un rôle prépondérant. L'un de nous l'a montré dans les bois et sciures [26] et il semble bien en être de même dans les litières (cf. p. ex. Lindeberg [24], Kendrick [18]).

Il nous a donc paru intéressant de connaître le rôle que pouvaient jouer les espèces dégradant la lignine, dans la formation de l'humus, rôle qui peut être direct ou indirect. On sait, en effet (Henderson [13]) que l'attaque d'un bois par un lignivore (pourriture blanche) libère

des aldéhydes aromatiques qui peuvent être utilisés par de nombreux microorganismes de la flore zymogène (Henderson et Farmer [15], Knösel [17]) avec ouverture du noyau benzènique (Henderson [14]), mais qu'ils pourraient aussi éventuellement être "neutralisés" par condensation sous forme de composés analogues aux substances humiques (Gadd [12]).

2. MATERIELS ET TECHNIQUES

Nous avons utilisé pour nos essais de la sciure de bois, en premier lieu parce que nos recherches antérieures ont été consacrées pour la plupart à l'écologie des champignons lignicoles, mais aussi parce que le tissu ligneux, presqu'uniformément incrusté de lignine constitue un milieu moins hétérogène qu'une paille, par exemple, où voisinent parenchyme et sclérenchyme.

Après avoir analysé les caractéristiques chimiques et les populations microbiennes des tas de copeaux mentionnés au début de ce travail * nous avons cultivé sur sciure fraîche, seules ou en combinaisons définies, diverses espèces isolées pour la plupart de ces tas. Enfin, nous avons procédé à des ensemencements massifs à l'aide de fragments déjà humifiés.

Mais nos premiers essais, mettant en jeu notamment *Bolbitius vitellinus*, *Penicillium* sp., *Mollisia cinerella* ** – dont nous avions montré l'activité en milieu liquide [28] – associés ou non entre eux et avec divers mélanges bactériens naturels, se sont révélés entièrement négatifs. Nous avons donc supposé que la matière ligneuse devait être soumise à de profondes transformations avant que des substances humiques y apparaissent. Nous avons alors eu recours à des cultures de:

1°) *Trechispora* sp.

2°) *Cytosporella* sp.

3°) deux associations naturelles dont nous préciserons ci-dessous la composition.

A. *Origine des cultures*

1) *Trechispora* sp. a été isolé de copeaux entreposés sur sol calcaire depuis

* Cette étude fera l'objet de publications séparées.

** Forme conidienne désignée à tort sous le nom de *Phialophora fastigiata* dans notre travail de 1957 [28].

7 — 8 ans. Au moment de leur analyse, la région où cette espèce se développait présentait un pH de 4,2 et renfermait 1,89% d'acides humiques et 4,18% d'acides fulviques. La souche obtenue provoquait une pourriture blanche caractéristique, assez active. Elle fournissait un test tyrosinase + et laccase faiblement +. Des essais préliminaires devaient nous montrer qu'elle se développait vigoureusement sur les seuls milieux acides.

2) *Cytosporella* sp. a été isolé de la même région que l'espèce précédente. Il forme un mycélium fuligineux bien développé et donne des tests tyrosinase faiblement + et laccase —. La croissance est assez bonne sur milieu à la cellulose. Comme bien des Sphéropsidales stromatiques, ce champignon est probablement un représentant des stades de dégradation du bois en l'absence de lignivore (stade *Melanomma*) ce qui ne l'empêche pas de se développer de façon satisfaisante en association avec *Trechispora*, comme nous le verrons plus loin.

3) Un premier inoculum mixte (no. 2) provient d'une culture massive âgée de 7 mois. Celle-ci avait été obtenue en ensemençant à l'aide de copeaux très altérés et prélevés dans la même région que les espèces précédentes, un mélange stérile et humidifié de 100 g de sciure fine (Tamis 90) et 5 g de $CaCO_3$. Au moment de son utilisation comme inoculum, le milieu est recouvert d'une croûte noire compacte, épaisse d'environ 3 mm et peuplée de nombreux Collemboles. Des fragments de cette couche ont été broyés aseptiquement dans l'eau et la suspension homogène ainsi obtenue a servi, d'une part à ensemencer nos fioles de sciure, d'autre part à une analyse microbiolgique.

La recherche des fixateurs d'azote atmosphérique sur milieu liquide électif montre l'absence d'*Azotobacter*, la présence de *Clostridium*, groupe acéto-butyrique. Par dilution sur plaques de gélose au Maltéa on a obtenu de très nombreuses colonies bactériennes et des champignons: *Fusarium* sp., *Penicillium* sp., *Graphium* sp. en grand nombre et, d'autre part, *Gliomastix convoluta* (Harz) Mason, *Mucor* sp., *"Stemphylium"* sp. et divers myceliums stériles hyalins.

4) Un autre inoculum mixte (no. 5) est constitué par des copeaux de même origine que les matériels précédents, mais prélevés immédiatement avant le début de l'expérience. En outre, les fioles ont été ensemencées en introduisant directement dans chacune une parcelle de produit; il en est résulté une certaine variabilité des cultures. D'autre part une suspension a été préparée comme dans le cas précédent et analysée, ainsi qu'une fiole de sciure obtenue par repiquage d'une culture massive de 6 mois. Nous n'y avons pas trouvé de fixateurs d'azote; par contre elles ont montré de nombreuses espèces bactériennes (Eubactéries, Proactinomycètes, *Polyangium* sp.) ainsi que *Streptomyces* spp. et divers champignons: *Mammaria echinobotryoïdes* Ces., *Graphium penicillioïdes* Cda., *Sporocybe* sp., *Stysanus stemonitis*, *Fusarium* sp. et un Basidiomycète, récemment apparu et encore à l'étude.

B. *Techniques de culture*

On introduit dans des fioles d'Erlenmeyer de 150 cc 8 grs de poudre de bois de hêtre, passant entre les tamis 20 et 80. Le milieu présentant un pH de

l'ordre de 5,5 convient pour les cultures de *Trechispora* et de *Cytosporella;* pour les inoculums mixtes, on le neutralise en mêlant intimement à la sciure 5% de $CaCO_3$ (pH du milieu préparé de l'ordre de 7,5).

Les fioles, bouchées au coton, sont stérilisées à l'autoclave à 115° C pendant 30 minutes. On leur ajoute ensuite aseptiquement 20 cc de la solution suivante, préalablement stérilisée:

Peptone de caséine "Roche"	6,0 g
Phosphate disodique	0,5 ,,
,, monopotassique	0,5 ,,
Sulfate de magnésium crist.	0,5 ,,
Chlorure de potassium	0,5 ,,
Eau ordinaire qsp.	1 litre

L'humidité est maintenue à une valeur suffisante par des apports périodiques d'eau distillée stérile, sauf dans le cas de *Trechispora* où l'attaque du bois s'accompagne de la production d'une grande quantité d'eau.

Après 6 mois, certaines fioles sont vidées de leur contenu dont le pH est mesuré immédiatement au potentiomètre. Puis on réunit les échantillons ayant subi le même traitement et on les laisse sécher à l'air libre à 25–30° C pendant 24 — 48 h. On sait en effet qu'une dessication trop poussée modifie les résultats des dosages de substances humiques. Le mélange est ensuite broyé et tamisé de manière à le rendre parfaitement homogène, puis soumis à l'analyse.

Les fioles mises en réserve sont alors l'objet de traitements variables suivant les cas et que nous indiquerons en détail plus loin. Ils ont été déterminés par les considérations suivantes: la stérilisation à l'autoclave détruit en partie et de façon imprévisible les composés humiques formés et d'autre part l'introduction d'une espèce dans un milieu déjà colonisé par une autre espèce aboutit à un échec à moins qu'elle ne s'accompagne d'une modification convenable des caractères de ce milieu. Nos essais préliminaires nous avaient montré que les inoculums mixtes et *Cytosporella* s'accommodaient bien d'un pH voisin de la neutralité, alors que celui-ci était défavorable à *Trechispora.* Nous avons donc apporté, suivant l'espèce à introduire soit 2 gouttes de H_3PO_4 pur, soit, 0,40 gr de $CaCO_3$, sous forme de bouillie stérilisée. Les fioles ainsi traitées sont ensemencées avec l'espèce choisie, mises à incuber pendant 4 — 5 mois, puis extraites et traitées comme précédemment.

C. *Méthodes d'analyse chimique*

1. Acides humiques et fulviques: Nous avons utilisé la méthode Chaminade (cf. Duchaufour, Traité de Pédologie, 1956, Annexe III p. 280) comportant l'extraction partielle du produit (10 g) par macération dans une solution de NaF à 1% (100 cc). Cependant, nous n'avons procédé à aucune décalcification préalable et comme nos sciures en fiole, à la différence des terres, ne sont jamais soumises au lessivage, nous comptons nécessairement, avec les acides fulviques, les substances réductrices hydrosolubles qui

se forment, parfois en grande quantité, au cours des expériences. Nous avons constaté, en effet, sur des essais parallèles, que dans le cas des lignivores (*Trechispora, Trametes rubescens, Phlebia aurantiaca*) ces substances hydrosolubles constituent entre les 2/3 et les 3/4 du chiffre total. Cette fraction n'est d'ailleurs pas dépourvue d'intérêt. Elle correspond, pour partie, à la fraction F_1 de la méthode Tiourine, modifiée par Duchaufour [12] et l'on conçoit aisément qu'en milieu acide, certains composés solubles, peut-être des acides organiques – de l'acide oxalique par exemple, que divers lignivores produisent en abondance – puissent avoir une action complexante et dissolvante sur les éléments minéraux.

Par contre le lessivage préalable ne modifie pas le chiffre des acides humiques bien que, dans la nature, une partie de ces derniers se trouve sous forme extractible à l'eau.

Le liquide obtenu par macération de 14 heures est additionné de 10% de H_2SO_4 pur pour séparer les fractions fulvique et humique et les dosages sont effectués par manganimétrie. Les résultats sont exprimés en milligrammes pour 100 g de matière sèche à 105° C dans le cas des acides humiques (en admettant que 1 cc $KMnO_4$ $N/10$ corresponde à 1,02 mg) et en centimètres cubes de $KMnO_4$ $N/10$ par gramme de matière sèche dans le cas des acides fulviques.

2. Lignine: La méthode employée est celle du De Forest Product Laboratory (cf. Levy-Hulot, L'analyse immédiate des bois, 1946, p. 218) modification de la méthode classique de Klason. La sciure, sans extraction préalable aux solvants organiques, est attaquée d'abord par l'acide sulfurique à 72% à froid pendant 16 heures, puis à l'ébullition pendant 4 heures après dilution de l'acide à 3%. Le résidu de lignine est recueilli sur verre fritté, lavé à fond à l'eau chaude, séché, pesé; enfin, le taux de cendres est déterminé sur une fraction aliquote. Nous employons pour ce dosage le résidu de l'extraction au fluorure après l'avoir amené à l'état de poudre fine (passant au tamis 80) à l'aide d'un microbroyeur.

Il nous a semblé essentiel de renoncer à l'extraction préalable aux solvants organiques, ces derniers pouvant entrainer des quantités appréciables de lignine – plus ou moins transformée – surtout dans le cas d'un bois déjà altéré.

Les résultats sont exprimés en pourcentage de matière sèche à 105° C; en outre nous avons calculé un indice de lignine L – mesurant le pourcentage de cette substance conservé dans le milieu – déterminé par la formule:

$$L = \frac{\text{Taux final de lignine} \times \text{poids final des solides totaux}}{26 \times \text{poids initial des solides totaux}} \times 100$$

Le chiffre 26 correspond au taux initial de lignine dans nos sciures.

3. Capacité totale d'échange: (cf. Duchaufour, *loc. cit.* 1956) Les échantillons sont soumis à macération puis percolation par l'acétate d'NH_4 N à pH 7. Après lavage à l'alcool à 96%, NH_4 est déplacé par NaCl 0,5 N, puis titré après distillation (Parnas-Wagner) en présence d'indicateur de Taschiro. Les résultats sont exprimés en milliéquivalents par 100 g de matière sèche à 105° C.

RÉSULTATS EXPÉRIMENTAUX

Les résultats des analyses chimiques sont réunis dans le tableau récapitulatif ci-dessous et leur examen appelle les remarques suivantes:

1°) L'activité de *Cytosporella* sur sciure est très réduite, sa croissance restant d'ailleurs pauvre. Après 6 mois, le bois a conservé sa structure initiale, avec particules distinctes, sans agrégats notables. La teinte seule a changé en passant au gris-brun; odeur fongique agréable. Quatre fioles ont été utilisées pour les analyses: leur pH final variait entre 5,0 et 5,1.

TABLEAU RECAPITULATIF

Caractéristiques des diverses sciures								
Inoculum	Durée en mois	pH final	Poids final	Acides humiques (g %)	Acides fulviques (cc/g)	Lignine %	Indice de lignine	T (me/100 g)
Cytosporella	6	5,0	—	0,15	14,74	26,13	—	12,1
	11	4,85	6,45	0,06	22,8	29,68	96	16,7
Trechispora	6	2,8	5,4	1,46	94,5	17,33	45	14,85
	11 *	5,0	4,53	2,46	113	—	—	—
Cytosporella	6							
+	+	3,2	4,29	3,96	131	20,71	43	25,43
Trechispora	5 **							
Trechispora	6							
+	+	8,5	3,57	3,25	98,8	22,77	39	—
Cytosporella	5 *							
Mixte no. 2	6	6,9	6,8	0,76	9,15	30,05	93,4	21,66
	11	6,76	4,96	0,85	25	35,34	85,5	37,48
Mixte no. 2	7							
+	+	3,4	4,72	2,46	68	32,36	70	40,81
Trechispora	4 **							
Mixte no. 5	6	7,2	—	1,26	9,14	29,44	—	24,7
Témoin.	—	5,5	—	0,11	12,67	25 à 26	—	9,7 à 10,2
Zone à *Trechispora* (dans la nature)	—	4,2	—	1,89	41,8	—	—	—

* Addition après 6 (ou 7) mois de $CaCO_3$

** ,, ,, ,, ,, ,, de H_3PO_4.

A ce moment, deux des fioles restantes reçoivent 5 cc de solution de peptone à 6 ‰; après 11 mois, la sciure n'est guère plus altérée, bien que sa teinte soit un peu plus sombre. Le pH s'est légèrement abaissé et une petite quantité d'acides fulviques s'est formée tandis

que la capacité d'échange augmentait un peu. Enfin la lignine ne parait pas attaquée, son indice s'établissant à 96 pour cent.

2°) Sous l'action de *Trechispora*, la sciure prend, en 6 mois, une teinte jaune pâle et répand une odeur agréable de chanterelle. Le milieu est très humide avec parfois une petite quantité d'eau libre au fond de la fiole. Les particules sont à peine visibles et l'ensemble s'est aggloméré en nodules compacts durcissant au séchage et se réduisant en poudre fine par simple trituration au mortier. Le pH mesuré sur 6 fioles varie entre 2,7 et 2,88. En présence de NaOH ou de $CaCO_3$, la coloration du produit passe au brun-rouge et fait retour au jaune-pâle par acidification. La liqueur d'extraction à l'eau est jaune-brun foncé et laisse déposer par addition d'alcool un abondant précipité gommeux; elle reste toutefois encore légèrement colorée, tandis qu'elle précipite et se décolore entièrement par le sous-acétate de plomb. Comme on pouvait s'y attendre, le taux de lignine est réduit et son indice montre que plus de 50 pour cent en ont été détruits. En même temps se forment des quantités importantes de substances réductrices hydrosolubles ou analogues aux acides fulviques et humiques.

Après 6 mois, deux fioles sont additionnées de 5 cc de solution de peptone à 6‰ et de 0,40 g de $CaCO_3$, puis maintenues en culture pendant 5 autres mois. Le milieu qui a viré par neutralisation partielle et pris une teinte brun-rougeâtre ne parait plus se transformer et, en tous cas, on ne note plus de croissance visible du champignon. Cependant, en fin d'expérience, on constate que le poids a encore diminué de façon appréciable et que de nouvelles quantités de substances humiques se sont formées.

Mais cet humus, qu'il ait été élaboré avant ou après l'apport de $CaCO_3$ est du type acide et rappelle celui des podzols: les acides humiques y ont une faible densité optique, proche de celle des acides fulviques et la capacité d'échange est à peine plus élevée que dans la sciure fraîche.

3°) Après 6 mois de culture, deux fioles attaquées par *Trechispora* reçoivent peptone et $CaCO_3$ comme ci-dessus et sont inoculées à l'aide de *Cytosporella*. En même temps, trois fioles de culture de cette dernière espèce reçoivent 5 cc de solution de peptone et sont ensemencées à l'aide de *Trechispora*. Ces cultures croisées ont donc été menées dans des conditions bien différentes et montrent après 11 mois des aspects en quelque sorte opposés: dans le premier cas,

la sciure, en partie neutralisée avant la réinoculation, présente un pH final de 8,5; les agrégats formés pendant la première période se sont transformés en quelques grains compacts, bruns et couverts d'un important feutrage gris-clair indiquant une croissance luxuriante de la Sphéropsidale. Dans le deuxième cas, le milieu n'a pas reçu de $CaCO_3$ et son pH final varie de 2,9 à 3,4. La sciure brunie par *Cytosporella* est encore reconnaissable, mais elle a pâli et s'est couverte d'un lacis de cordonnets blancs appartenant à *Trechispora*.

Cependant, les résultats analytiques sont voisins: la décomposition est un peu plus avancée dans le premier cas, où *Trechispora* s'est développé seul un mois de plus; c'est l'inverse pour la synthèse des acides humiques et fulviques, mais il s'agit de chiffres du même ordre de grandeur et les acides humiques formés ont dans les deux cas des densités optiques égales et nettement supérieures à celles des acides de *Trechispora*.

4°) Sous l'action de l'inoculum mixte no. 2, la sciure a pris en 6 mois une teinte brun-noir et elle répand une odeur prononcée de fumier. La structure initiale est en grande partie détruite et le milieu tend à constituer des agrégats volumineux, durcissant en sèchant. Le pH mesuré sur 3 fioles varie de 6,84 à 7,02. Suivant le schéma classique, le taux de lignine s'élève, mais en même temps se forment de petites quantités d'acides humiques et la capacité d'échange double.

A ce moment, 3 autres fioles reçoivent chacune 5 cc de solution de peptone, puis on les conserve pendant 6 mois encore. A la fin de ce délai, la sciure est d'un noir intense et répand une odeur fruitée. On note une attaque appréciable de la lignine, malgré l'augmentation de son pourcentage; le taux d'acides fulviques et la capacité d'échange ont encore presque doublé, tandis que le chiffre des acides humiques se modifie peu. Or ces acides, formés par conséquent avant l'attaque de la lignine, ont une densité optique sensiblement plus élevée que dans les essais précédents.

5°) Deux fioles ensemencées depuis 7 mois par le même inoculum no. 2 sont additionnées de peptone et de 2 gouttes d'acide phosphorique puis inoculées par *Trechispora*. Ce dernier paraît tarder à s'installer et n'est pas visible 2 mois plus tard. Pourtant en fin d'expérience (11 mois), le milieu a pris une teinte plus claire, brun-jaune, reflétant l'activité du Basidiomycète. Le pH s'est abaissé à 3,6–3,2, et l'on note une légère diminution de la lignine (l'action du

champignon lignivore ne s'est exercée que 4 mois) tandis que la formation des substances humiques est exaltée et que la capacité d'échange s'élève, plus même qu'avec l'inoculum 2 seul. Enfin, la courbe des densités optiques est très voisine de celle obtenue à partir des traitements mixtes (cf. § 3°).

6°) L'essai à partir de l'inoculum mixte no. 5 n'a pas été poursuivi assez longtemps pour fournir des indications bien précises. Autant que l'on puisse dire, il semble que l'évolution soit tout à fait parallèle à celle que déterminait l'inoculum no. 2. En fin d'expérience, le pH des 6 fioles s'établit entre 6,74 et 7,56, les valeurs extrêmes correspondant à des échantillons de teinte sombre, brun-jaune foncé, et répandant, eux aussi, une odeur de fumier. Le contenu des autres fioles paraissait incomplètement transformé avec des zones noircies et d'autres de coloration normale.

L'analyse montre cependant une activité un peu supérieure à celle de l'inoculum n° 2; ceci peut s'expliquer par la présence d'un Basidiomycète probablement responsable de l'apparition des zones pâles que nous venons de signaler. Mais il s'agit d'une espèce à croissance lente, isolée depuis trop peu de temps pour que nous connaissions ses aptitudes.

CONCLUSIONS

1. Nous avons obtenu, *in vitro*, la transformation d'une même substance organique, de la sciure de hêtre, suivant deux schémas différents.

Dans la nature, sans doute, de nombreux facteurs gouvernent le déroulement de l'un ou l'autre de ces schémas. Dans nos expériences, seule intervient la teneur en bases, choisie en vue de favoriser la croissance de tel ou tel groupe de microorganismes. On peut supposer que, dans le sol, les facteurs écologiques déterminent la composition du peuplement microbien qui, à son tour, détermine le type d'humus formé.

2. Les transformations que nous observons dans les sciures ne sont pas essentiellement différentes de celles que d'autres auteurs ont décrites dans les litières (Mikola [30]).

Les Basidiomycètes lignivores, correspondant à notre stade *Leptoporus* [26], sont les destructeurs les plus actifs de la matière végétale et surtout de la lignine. Ils préfèrent – pour la plupart,

car il y a des exceptions – les milieux acides dont ils abaissent le pH; au contraire, les espèces ou associations non lignivores ne modifient pas celui-ci ou même élèvent sa valeur [27]. C'est ici le cas de *Cytosporella* et de l'inoculum n° 2.

La sciure fraîche, comme les litières, est d'abord attaquée par des espèces glucophiles (= sugar-fungi de Garrett), puis, quand les aliments facilement utilisables sont épuisés, d'autres formes plus spécialisées s'installent: espèces fuligineuses "bleuissant" les bois (*Phialophora*, Spheropsidales) ou lignivores décolorant le milieu. Nous ne savons pas ce qui commande le développement de l'un ou l'autre de ces groupes d'organismes (le pH est un facteur parmi beaucoup d'autres) mais ils peuvent se trouver étroitement associés dans le même substrat: Anstett [1] avait remarqué, dans un fumier artificiel de sarments de vignes, des poches rougeâtres qu'il expliquait par des considérations de pH et de rH_2 et qui étaient sans doute liées à la présence de lignivores, mais nous ne pouvons pas dire si l'acidité et le caractère réducteur du milieu étaient la cause ou la conséquence de leur développement.

Quoiqu'il en soit, dans nos expériences, les populations basophiles ou neutrophiles fournissent des produits de teinte sombre où prédominent les acides humiques à capacité d'échange élevée. Les espèces acidophiles fournissent en grande quantité des produits de teinte claire, de densité optique et capacité d'échange faibles.

3. La flore neutrophile est représentée dans nos essais par l'inoculum n° 2. La dégradation de la sciure est lente et les acides humiques sont produits surtout au cours des six premiers mois à un moment où la lignine ne subit aucune dégradation – dans la mesure où les méthodes classiques de dosage permettent de le savoir –. Au contraire, lorsque le taux de cette substance diminue, par un mécanisme que nous n'avons pas étudié, la teneur en acides humiques n'augmente plus de façon significative. Il s'agirait donc ici, conformément aux vues de Wittich [36] de produits de synthèses microbiennes, élaborés vraisemblablement par l'intermédiaire de pigments quinoniques, suivant des processus aujourd'hui bien connus.

4. La flore acidophile est représentée dans nos expériences par *Trechispora*. La lignine est décomposée plus rapidement que les autres constituants du bois, et, en même temps, se forment des quantités considérables de substances réductrices d'apparence

mucilagineuse, les unes hydrosolubles et analogues probablement aux polysaccharides de Bernier [4], les autres dispersables dans les solutions de FNa, comme les composés humiques. Rappelons d'ailleurs que l'on a souvent noté l'accumulation, dans le sol, en même temps que la lignine en disparaissait, de grandes quantités d'uronides (Tepper [34]) ou plutôt de substances décarboxylables (cf. Deuel *et al.* [9])

On sait d'autre part que les acides humiques comportent une fraction hydrolysable, composée principalement de chaines carbonées et de restes peptidiques et une fraction non hydrolysable de nature aromatique. La première, selon Mayaudon et Simonart [29] est élaborée surtout à partir des glucides, la seconde à partir de la lignine. Or les acides humiques de *Trechispora* paraissent assez fragiles puisque la simple stérilisation de la matière sèche à l'autoclave en détruit près de 50 pour cent. Nous les comparerons à des "gommes" fongiques, plus ou moins condensées et renfermant une proportion réduite de restes aromatiques. Or Kononova [19] exprimait une opinion analogue à propos des acides humiques des podzols.

Comme on l'a souvent remarqué, les acides humiques pâles et les acides fulviques se forment en même temps que la lignine disparait et l'on en avait conclu que cette substance était progressivement incorporée à l'humus par l'intermédiaire de complexes humo-lignine. Mais cette opinion ne parait plus guère admise (Henin [16]) notamment depuis le travail de Bremner (*loc. cit.*) montrant que l'oxydation de substances humiques par le nitrobenzène alcalin libère de très faibles quantités de ces aldéhydes aromatiques que fournit la lignine dans les mêmes conditions. Morrison [31] en a obtenu davantage à partir de certaines fractions humiques, mais ceci ne modifie pas sensiblement les données du problème. On sait en effet que la lignine, surtout en milieu acide, est attaquée par les lignivores et fragmentée en unités simples de nature aromatique utilisables par d'autres microorganismes (schéma de Henderson). L'action de ces derniers est probablement conforme au cycle classique (cf. Arnaudi *et al.* [2]) comportant le raccourcissement des chaines latérales et l'ouverture du cycle par formation d'orthodiphénols. Même si nous faisons abstraction des commensaux des lignivores, nous retrouvons les expériences de Gadd [12] suivant lesquelles les unités aromatiques seraient à

nouveau polymérisées sous l'influence de la laccase pour former des substances solubles analogues aux composés humiques. Ainsi, dans tous les cas, la lignine serait détruite avant d'être incorporée à l'humus.

5. On admet que dans le sol on a affaire, le plus souvent, à des mélanges, en proportions différentes suivant les cas, de produits plus ou moins condensés. En fait, la chromatographie sur colonne d'amidon a permis à Kononova *et al.* [21] de reconnaître la présence, dans les substances humiques, de trois fractions dont les importances relatives varient en sens inverse des acides fulviques aux acides humiques des tchernozems, en passant par les acides humiques des podzols. L'action successive de l'inoculum n° 2 et de *Trechispora* entraîne ainsi la formations de composés intermédiaires qui sont, à notre avis, le résultat d'une superposition des produits élaborés par les deux participants.

Dans le cas de *Trechispora* puis *Cytosporella* – ou inversement – les deux espèces constituent entre elles une synergie. L'action du lignivore parait conditionner absolument celle de la Sphéropsidale et les deux espèces ensemble sont plus actives vis-à-vis de la lignine, produisent plus d'acides humiques que *Trechispora* seul. Enfin, les valeurs les plus élevées de la capacité d'échange se rencontrent dans les inoculations croisées. On peut supposer que le milieu, colonisé d'abord par l'espèce lignivore contient des aliments utilisables par *Cytosporella*, gommes ou composés aromatiques; ceci correspondrait à la légère élévation du taux de lignine que nous enregistrons malgré la baisse de l'indice. Il est plus difficile d'expliquer pourquoi la sciure envahie par *Cytosporella* permet à *Trechispora* de manifester une activité supérieure à celle d'une culture pure.

RÉSUMÉ

On a cultivé sur sciure de hêtre additionnée de peptone et de sels minéraux un Basidiomycète déterminant une pourriture blanche, une Spheropsidale "bleuissant" le bois et deux inoculums naturels mixtes. Le Basidiomycète acidifie le milieu, dégrade la lignine et produit une grande quantité de composés humiques pâles. Les inoculums naturels décomposent peu la lignine et élaborent des composés humiques sombres. Ces inoculums, ainsi que la Sphéropsidale, en culture mixte avec le lignivore fournissent un humus de type intermédiaire et la capacité d'échange s'élève fortement.

Ces résultats sont discutés en fonction de la littérature récente.

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LETTER TO THE EDITORS

Providing aseptically cultivated plants with water through bacteria-tight glass-filters

For many investigations, raising plants under aseptic conditions is a prerequisite, and many efforts have already been made to solve this problem (Gerretsen [1], Sobieszczanski [2], Nilsson [3] and others). For some problems only the roots need be kept under aseptic conditions, allowing the tops to grow freely in the air. Gerretsen [1] developed a system for such a culture in which the soil to be maintained sterile is covered by protecting layers of paraffined and of silver-coated (catadyne) sand. This system proved to be successful and very reliable. It has only one disadvantage: the growing plant must be provided with water and nutrient salts from a flask which must be sterilized together with the culture vessel and tightly connected with it by glass tubing. This makes the whole equipment rather complicated and difficult to handle. A much simpler set-up is of course possible, adding the water or nutrient solution periodically through a tube inserted into the soil around the plant and plugged with cotton-wool. However, since the cotton-plug must be removed many times, the substrate is liable to be contaminated fairly readily.

To avoid complications associated with the connections to the large flask and, at the same time, to prevent ready contamination, the use of bacteria-tight glass filters is emphasized.

The arrangement is shown schematically in Fig. 1.

1. Culture vessel (a milk-bottle can be used after cutting off it's bottom).
2. Sand coated with elementary silver (catadyne).
3. Sand coated with paraffin.
4. Ground glass joint.
5. Transparent polyvinyl tubing.
6. Bacteria-tight glass filter.
7. Soil.
8. Layer of glass wool.
9. Glass cap during sterilisation and germination.
10. Cotton-wool.

The procedure for starting an experiment can briefly be described is follows: The apparatus in Fig. 1 is assembled and filled with the required soil or other culture medium. It is recommended that the medium be previously sterilized so as to reduce the time required to sterilize the assembled apparatus. Sufficient water is now added to bring the soil to the right

moisture status and the glass cap is then placed on top of the vessel. The space between the cover and the vessel must be plugged with cotton-wool. The

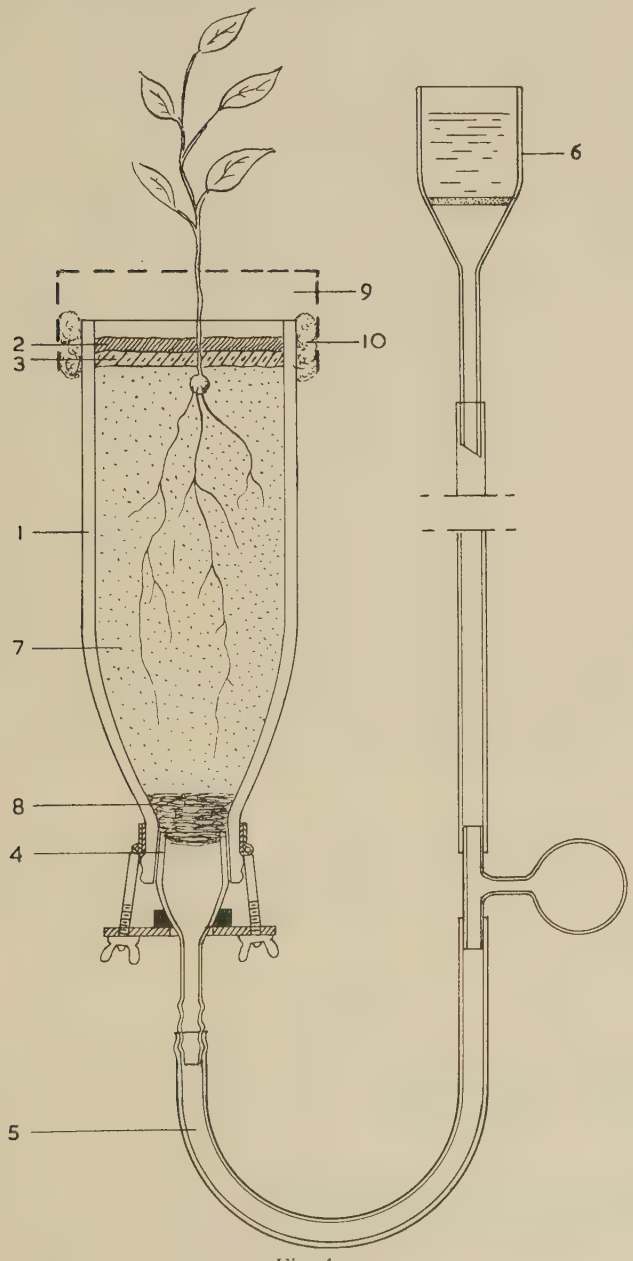

Fig. 1.

lower part of the culture-vessel must contain some water; the glass joint, the whole of the tubing connecting with the filter as well as the space in the latter beneath the filter disc must also be completely filled with water, allowing no gas bubbles. To prevent gas formation during sterilisation, the filter is placed vertically upside-down in a beaker of warer. Any gas formed in the system between the filter disc and the soil in the container (1) can then rise and escape through the soil, thus avoiding gas locks which could prevent the correct capillary suction of the liquid from the filter to the soil. The whole apparatus is sterilized by autoclaving in this position. After cooling, the cap (9) is lifted for a moment, under as strictly aseptic conditions as possible (in a special room, sterilized by UV light or by any other means, and working as fast as possible) and the pre-sterilized and germinated seed(s) are planted in the soil. The glass cap is now immediately replaced, and the apparatus placed in full light for the development of the plants. As soon as the latter are tall enough (nearly reaching the cap), the glass cap is removed and replaced by the protecting sand layers (3) and (2), spreading them around the stem of the plant. This manipulation again must be performed with all possible precautions against contamination. The filter (6) is now is taken out of the beaker, fixed in the erect position, and filled with water or with the required solution, which need not be sterilized.

The capillary action of the soil ensures an adequate uptake of the water through the filter disc. By displacing the filter up or down the flow of the solution to the container, and consequently the moisture content of the soil, can be regulated.

Careful work and the use of high-quality, bacteria-tight filter crucibles quarantee an entirely sterile medium for the development of the roots.

This arrangement has been succesfully used by the author for growing tomato plants in experiments involving the inoculation of the soil with cultures of microbes breaking down substances containing organic phosphorus[4].

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Received May 20, 1959.

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